Soil microbial community structure and function of agriculturally used Mollisols in the periurban area around Buenos Aires, Argentina, with emphasis on pesticide and heavy metal contamination

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This work is dedicated to

Johann Schindele † (RIP)

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Zusammenfassung

Buenos Aires, die Hauptstadt von Argentinien, ist umgeben von einem landwirtschaftlichen Grüngürtel mit fruchtbaren Mollisolen, welcher in erster Linie den gesamten Bedarf speziell an Gemüse für die städtische Bevölkerung bereitstellt. Die schnell wachsende Bevölkerung dieser Megastadt verursacht einerseits eine Verringerung der landwirtschaftlich nutzbaren Fläche und andererseits eine höhere Nachfrage an Nahrungsmitteln. Um genügend Nahrungsmittel zu produzieren, sind Intensivierung der Landwirtschaft und Erhaltung nachhaltiger Bodenfruchtbarkeit von essentieller Bedeutung. Dennoch ist die Bevölkerung durch eine gesteigerte Anwendung von Pestiziden und schwermetallhaltigen Düngern, Eutrophierung wegen hoher Dungausbringung und möglicher Bodendegradation zunehmend über die negativen Aspekte dieser landwirtschaftlichen Intensivierung wie z. B. Umweltverschmutzung besorgt, weswegen organische Bewirtschaftungsmaßnahmen mehr und mehr an Aufmerksamkeit gewinnen. Die innere Zone des Grüngürtels wird vielfältig landwirtschaftlich genutzt. Neben Brachen und Weiden werden konventionelle und organische Bewirtschaftung auf Äckern und unter Gewächshäusern betrieben, wobei noch anthropogen unbeeinflusste Flächen existieren, deren Böden eine Referenz des natürlichen Bodenzustandes darstellen. Verschiedene Landnutzungssysteme und unterschiedliche landwirtschaftliche Praxis, die potenziell die Bodenökologie und –umgebung verändern können, haben starken Einfluss auf mikrobielle Gemeinschaften. Bodenmikroorganismen sind nicht nur äußerst wichtig für die Fruchtbarkeit und Nachhaltigkeit, sie spielen außerdem eine wesentliche Rolle in globalen Nährstoffkreisläufen.

Ziele dieser Dissertation waren, zu untersuchen, ob unterschiedliche landwirtschaftliche Praxis mit variierendem Eintrag von Pestiziden und Schwermetallen Bodenverschmutzung verursachen und ob die verschiedenen Landnutzungssysteme zu einer Veränderung der bodenmikrobiologischen Struktur und Funktion führen. Außerdem wurde, geprüft ob eine intensive konventionelle Bewirtschaftung zu einer Verschlechterung der Bodenqualität führt und ob deren Ersatz durch organische Bewirtschaftung diese Situation verbessern kann. Es wurde ebenso untersucht, ob eine Umwandlung der Landnutzung, ausgehend von einer Brache hin zu typischen Landnutzungssystemen des Untersuchungsgebietes, die bodenmikrobiologische Struktur und Funktion beeinflusst, wobei spezielle Schwerpunkte auf Pestizidapplikation als auch auf konventionelle sowie organische Bewirtschaftungsweisen

gelegt wurden. Daher wurden typische Landnutzungssysteme untersucht sowie ein Feldexperiment über die Dauer einer kompletten Vegetationsperiode von neun Wochen durchgeführt, beginnend mit einer Brache, welche sowohl gepflügt wie auch ungepflügt die natürliche Variabilität zeigte. In diesem Experiment wurden konventionelle Behandlungen unter Verwendung mineralischer Dünger sowie durch das Insektizid Endosulfan und das Fungizid Chlorothalonil allein wie auch in Kombination simuliert, während organische Behandlungen unter Verwendung von Humus sowie durch das biologische Insektizid *Bacillus thuringiensis* und das Fungizid Kupferoxychlorid ebenfalls allein und in Kombination nachempfunden wurden.

In Oberböden der verschiedenen Landnutzungssysteme konnten außer den Insektiziden Cypermethrin, Deltamethrin, Malathion und Triazofos, welche unter der Bestimmungsgrenze (0,1 μg kg^{-1}) lagen, die Insektizide Carbofuran, Chlorpyrifos, Dimethoat, Endosulfan und Permethrin, das Fungizid Chlorothalonil und das Herbizid Trifluralin zwischen 0,2 und 34,2 μg kg^{-1} ausschließlich in konventionell bewirtschafteten Böden von Äckern, unter Gewächshäusern und in einer Brache, die vorher auch konventionell bewirtschaftet wurde, quantifiziert werden. In den Böden der übrigen Landnutzungssysteme lagen alle dieser Pestizide unter der Bestimmungsgrenze. Die mittleren Schwermetallgehalte (Fe 17,1 – 18,9 und Mn 0,60 – 0,79 g kg^{-1}, Cu 16,8 – 19,2, Ni 6,8 – 7,4, Pb 10,6 – 13,2 und Zn 32,5 – 39,8 mg kg^{-1}) unterschieden sich nicht signifikant innerhalb der Landnutzungssysteme. Cadmium wurde nur in einer Brache detektiert (0,9 mg kg^{-1}). Demzufolge wurde die Kontaminationsgefahr ausgehend von Pestiziden und Schwermetallen als gering bewertet. Folglich wird kein alarmierendes Risikopotential für menschliche Gesundheit, Boden– und Wasserqualität, Pflanzenwachstum und Tierwelt erwartet.

Für die Charakterisierung der bodenmikrobiologischen Struktur und Funktion in Böden der verschiedenen Landnutzungssysteme wurden zwanzig Phospholipidfettsäuren (PLFS) bzw. folgende Parameter analysiert: Enzymaktivitäten (saure Phosphatase, Arylsulfatase, Cellulase, Dehydrogenase und Urease), basale und substrat-induzierte Respiration, bodenmikrobielle Biomasse, metabolischer Quotient, netto-Stickstoff-Mineralisation, netto-Nitrifikation und potentielle Denitrifikation. Je eine Hauptkomponentenanalyse (HKA) wurde für die funktionellen und strukturellen Parameter durchgeführt, um die Datenmenge zu reduzieren und um korrelierende Parameter zu vereinigen. Für die funktionellen

Parameter wurden vier Hauptkomponenten (mikrobielle Kapazität, Mineralisationsaktivität, Stickstofftransformationspotential und metabolische Aktivität) extrahiert, während für die PLFS sechs mikrobielle taxonomische Gruppen unterschieden werden konnten.

Mit Hilfe einer anschließenden Diskriminanzanalyse, berechnet durch die Faktorwerte der HKA für die Funktionsparameter, wurden sechs unabhängige Landnutzungsgruppen differenziert. Fast alle Böden der konventionell bewirtschafteten Gewächshäuser sowie der organisch bewirtschafteten Gewächshäuser und Äcker, Weiden und des Referenzstandortes konnten den erwarteten Landnutzungsgruppen zugeordnet werden. Dabei war ein Wechsel zwischen Innen- und Außenbewirtschaftung im organischen Landbau erkennbar. Böden konventionell bewirtschafteter Äcker und Brachen wurden in einer Gruppe vereinigt, was von einer hierarchischen Klassenanalyse bestätigt wurde und stärkste Ähnlichkeit zwischen diesen beiden Landnutzungssystemen zeigte. Außerdem demonstrierte die Klassenanalyse Ähnlichkeiten zwischen konventionellen und organischen Freiland- sowie konventionellen und organischen Gewächshausbewirtschaftungen. Die Referenz- und Weideböden bildeten zwei weitere eigenständige Klassen im Vergleich zu den verbleibenden Landnutzungssystemen, da sie deutliche Unterschiede zu jenen der Anbauflächen aufwiesen. Jegliche Landnutzungsänderung führte zu einer starken Reduktion der basalen Respiration und des metabolischen Quotienten verglichen mit den Referenzböden, während die Weideböden eine deutliche Steigerung in ihrer Biomasse und ihren Enzymaktivitäten erkennen ließen. Es konnten jedoch keine signifikanten Unterschiede zwischen den Bewirtschaftungsweisen und der Innen- und Außenbewirtschaftung gefunden werden.

Im Zuge landwirtschaftlicher Nutzung zeigten alle taxonomischen Mikroorganismengruppen, die durch die PLFS-Analyse erhalten wurden, eine Biomassereduktion relativ zu den Referenzböden. Die niedrigste Biomassereduktion wurde in Bracheböden aufgrund des Eintrags organischen Materials durch Exkremente des Tierbestands detektiert, während Ackerböden höhere PLFS-Gehalte aufwiesen als jene unter Gewächshäusern. Verglichen mit den konventionell bewirtschafteten Böden wurden in jenen organischer Bewirtschaftung höhere Gehalte Gram-negativer Bakterien und Pilze ermittelt. Weitere Effekte hinsichtlich der Bewirtschaftungsweisen konnten nicht gefunden werden. Im Allgemeinen zeigten Bracheböden die niedrigsten Gehalte der taxonomischen Gruppen, was darauf hindeutete, dass Bodenmikroben lange brauchen, um sich von den Folgen landwirtschaftlicher Nutzung

zu erholen. Die relativen Anteile der mikrobiellen Gruppen in Böden der anderen Landnutzungssysteme resultierten in deutlich niedrigeren Werten für Actinomyceten, Gram-positive, anaerobe und speziell Gram-positive, aerobe Bakterien im Vergleich zu den anthropogen unbeeinflussten Böden. Nur in Weideböden hatten die Actinomyceten niedrigere Werte als die der Referenz. Mit Ausnahme geringerer Anteile von Protozoa in Böden konventionell bewirtschafteter Gewächshäuser und Brachen verglichen mit den Referenzböden, wurden in Böden aller Landnutzungssysteme für Protozoa, Pilze und Gram-negative Bakterien höhere Anteile gefunden, obwohl deren absolute PLFS-Gehalte reduziert wurden. Gram-negative Bakterien hatten sogar signifikant höhere Werte in Acker- und Bracheböden im Vergleich zu jenen der restlichen Landnutzungssysteme. Unterschiede zwischen den Bewirtschaftungsweisen sowie zwischen Freiland- und Gewächshausbewirtschaftung wurden nicht gefunden. Des weiteren war eine Differenzierung der Landnutzungssysteme durch eine Diskriminanzanalyse nicht möglich, während eine hierarchische Klassenanalyse die Ergebnisse der oben durchgeführten im Wesentlichen bestätigte.

Für die Auswertung des Feldexperiments wurden die selben Parameter wie oben beschrieben analysiert, um für die Untersuchung der verschiedenen Landnutzungssysteme die bodenmikrobiologische Struktur und Funktion bewerten zu können. Eine HKA wurde ebenfalls sowohl für die funktionellen als auch für die strukturellen Parameter durchgeführt, wobei fast die gleichen vier Hauptkomponenten, mikrobielle Kapazität, Mineralisationsaktivität, metabolische Aktivität und Stickstofftransformationspotential, für die funktionellen Parameter ermittelt wurden. Eine sinnvolle Klassifikation war jedoch für die analysierten PLFS wegen eines zu homogenen Datensatzes, gleicher Boden- und Klimabedingungen sowie erst kürzlich veränderter Bodenbehandlung nicht möglich.

Erneut wurde eine anschließende Diskriminanzanalyse, berechnet mit den Faktorwerten der HKA, für die funktionellen Parameter durchgeführt, welche deutlich zwischen Brache, konventionell und organisch bewirtschafteten sowie unbehandelten Parzellen unterscheiden konnte. Eine Unterscheidung zwischen Böden der selben Bewirtschaftungsweise hinsichtlich unterschiedlicher Pestizidapplikationen war jedoch ebenso wenig möglich wie hinsichtlich der Applikationsmenge. Dennoch kann gefolgert werden, dass eine Änderung der Landnutzung die Bodenfunktionalität beeinflusst und dass die Bestimmung dieser Parameter ein

potentielles Werkzeug zur Betrachtung von Bodengesundheit und der Umwandlung von Brachen in sowohl konventionell als auch organisch bewirtschaftete Äcker ist.

Die PLFS 14:0, 17:0 und 10Me17:0 sowie die mikrobiologisch taxonomische Gruppe der Protozoa, die durch die PLFS 20:4ω6 repräsentiert wird, reagierten sowohl in relativen als auch in absoluten Gehalten am sensibelsten auf die verschiedenen Düngergaben. Nach starken Schwankungen zeigte der PLFS-Summengehalt, der die gesamte lebende Biomasse repräsentiert, am Ende des Experiments geringere Werte in organischen, verglichen mit konventionellen Behandlungseinheiten. Wie bei der bodenmikrobiologischen Funktion wurden innerhalb der Struktur keine Effekte weder durch die Art noch durch die Menge der Pestizide verursacht. Außerdem war die Zugabe des Gram-positiven *Bacillus thuringiensis* durch die PLFS-Analyse nicht detektierbar. Allerdings war es möglich, mit Hilfe einer Diskriminanzanalyse, die mit den Faktorwerten aus der HKA der PLFS berechnet wurde, deutlich zwischen Böden der Brache-, organisch und konventionell bewirtschafteten sowie der unbehandelten Parzellen zu differenzieren, wobei die Böden der letzteren von denen der konventionell bewirtschafteten erst nach sechs Wochen nach Düngerzugabe voneinander zu unterscheiden waren.

Schließlich wurden in beiden Teilen dieser Dissertation signifikante Korrelationen zwischen den absoluten PLFS-Gehalten der einzelnen mikrobiellen taxonomischen Gruppen und den bodenmikrobiologischen Funktionsparametern gefunden, was auf enge Zusammenhänge zwischen bodenmikrobiologischer Struktur und Funktion hindeutet. Daher besitzt die Zusammensetzung der mikrobiellen Gesellschaft alleine noch keinen Indikatorwert und muss mit den Funktionsparametern in Verbindung gesetzt werden. Einzelne untersuchte Parameter konnten weder signifikante Unterschiede zwischen Böden verschiedener Landnutzungssysteme noch innerhalb des Feldexperiments aufdecken. Daher kann gefolgert werden, dass diese schluffigen und tonigen Mollisole der inneren Zone des Grüngürtels um Buenos Aires, die eine potentiell geringe Bioverfügbarkeit von Xenobiotika aufweisen, nicht sensibel auf eine intensive landwirtschaftliche Nutzung reagieren. Obwohl sich eine kurz- und mittelfristige Bodendegradation als Folge dieser Nutzung nicht zeigte, wie aus dem Feldexperiment und der Untersuchung der typischen Landnutzungssysteme hervorging, wird dringend geraten, die Langzeiteffekte weiter zu untersuchen.

Resumen

En Buenos Aires, la cápital de Argentina, la población en rápido crecimiento causa, por un lado, una disminución del sector agrario y, por otro, un aumento en el consumo de productos alimenticios. Esta metrópolis se encuentra rodeada por un cinturón verde agrícola, contituido por Mollisoles fertiles, el cual abastece por completo la necesidad, especialmente de verduras, de la población urbana. Para que la poducción alimenticia de abasto es de suma importancia la explotación agrícola y el mantenimiento de la sustentabilidad del suelo. Sin embargo, el aumento en el uso de pesticidas y abonos con metales pesados, la eutrofización a causa de la alta aplicación de fertilizantes, y una posible degradación de los suelos, a llevado a que la población se encuentre cada vez más preocupada por los aspectos negativos de esta explotación agrícola, tales como la contaminación. Por consiguiente estan ganando cada vez más resonacia los cultivos orgánicos. En la zona interna del cinturón verde se utilizan diferentes sistemas agrarios. Junto a barbechos y pastizales se practican sistemas de cultivo convencionales y orgánicos tanto en los campos de cultivo como bajo los invernaderos, y no dejan de existir áreas sin ningúna influencia antrópogenica, cuyos suelos se utilizarón como referencia para representar el estado natural de los suelos. Las comunidades microbianas se ven influenciadas fuertemente por quellos sistemas de uso del suelo y las diferentes practicas agrarias con potencial de modificar la ecológia del suelo y su entorno. Los microorganismos del suelo no sólo son importantes para la fertilidad y sustentabilidad de este, sino que también juegan un papel muy importante en los ciclos globales alimeticios.

Las metas de esta disertación eran, investigar si las diferentes practicas agrarias con respectivas variaciones en el uso de pesticidas y metales pesados causan la contaminación del suelo, y si los diferentes sistemas de cultivo llevan hacia una modificación de las estructuras sociales microbianas del suelo y sus funciones. Además se investigo si un manejo convencional tiene como consecuensia una disminución de la calidad del suelo, y si es posible mejorar esta situación suplantandolo por un manejo orgánico. También se examino si un cambio en el uso del suelo partiendo de un barbecho hasta llegar a un sistema típico de cultivo utilizado en el área a examina modifica la estrutura social microbiana y sus funciones. Al hacer esto se puso mayor enfasis en aplicaciones de pesticidas y en sistemas de cultivo convencionales como orgánicos. Para ello se llevo a cabo una investigación sobre los sistemas típicos de cultivo y se conceptuo un experimento de campo con la duración de un

periodo de vegetación completo, es decir nueve semanas. Se empezo por un barbecho, la cual muestra tanto estando arada como no la variabilidad natural. En este experimento se simularon aplicaciones convencionales, tanto individuales como en combinación, de fertilizantes minerales, del insecticida endosulfan y del fungicida chlorothanil. Para la parte orgánica se simularon igualmente, por separado y en combinación, aplicaciones de humus, el insecticida biológico *Bacillus thuringiensis* y el fungicida oxicloruro de cobre.

En el subsuelo de los diferentes sistemas de cultivo fue posible cuantificar a parte de los insecticidas cypermethrin, deltamethrin, mathion y triazofos, que se encontraron bajo el valor minimo de detección, únicamente en los suelos de los campos cultivados convencionalmente, en los situados bajo invernaderos y en los suelos de barbecho los siguientes insecticidas: carbonfuran, chlorpyrifos, dimethoat, endosulfan y permethrin, el fungicida chlorothalonil y el herbicida trifluralin, todos se encontraron representados entre 0,2 y 34,2 $\mu g\ kg^{-1}$. En los demás suelos procedentes de los otros sistemas de cultivo se encontro que estos pesticidas están bajo el valor minimo de detección. Dentro de los diferentes sistemas de cultivo, nose encontro ningúna diferencia significante entre los valores promedio de metales pesados (Fe 17,1 – 18,9 y Mn 0,60 – 0,79 $g\ kg^{-1}$, Cu 16,8 – 19,2, Ni 6,8 – 7,4, Pb 10,6 – 13,2 y Zn 32,5 – 39,8 $mg\ kg^{-1}$). En el barbecho se detecto cadmio (0,9 $mg\ kg^{-1}$). Consecutivamente se catálogo como leve el peligro de contaminación a partir del uso de pesticidas y metales pesados. Por lo tanto, no se cuenta con ningún riesgo potencialmente alarmante para la salud humana, la calidad del suelo y agua, el crecimiento de la vegetación y el mundo animal.

Para la caracterización de la estructura social microbiana del suelo y sus funciones dentro de los diferentes sistemas de cultivo se analisaron veinte fosfolípidos, ácidos grasos, (PLAG) y los siguientes parámetros: actividad enzimática, (fosfatase ácida, arilsulfatasa, celulasa, dehidrogenasa y ureasa), respiración de basales e inducia por sustrato, biomasa microbiana, cociente metabólico, mineralización de nitrógeno neto, nitrificación neto y denitrificación potencial. Con el fin de reducir la cuantidad de datos y unir parámetros condicionados se realizo por parámetro funcional y estructural un análisis del componente principale (ACP). Para los parámetros funcionales se extrajeron cuatro componentes principales (capacidad microbiana, efectividad de mineralización, potencial de transformación de nitrógeno y actividad metabólica) mientras que para los PLAG se pudieron identificar seis grupos taxonómicos microbianos.

Con la ayuda de un análisis discriminante, calculado a partir de los valores de factor de la ACP de los parámetros funcionales, se identificaron seis grupos independientes de uso de suelo. Casi todos los suelos de los invernaderos cultivados convencionalmente como también de los cultivados orgánicamente y de los campos, pastizales y puntos de referencia se ordenaron dentro del grupo de uso de suelo esperado. Al hacer esto, se noto una diferencia entre el cultivo orgánico a cielo abierto y el cultivo orgánico bajo invernadero. Suelos provenientes de cultivos conventionales y barbechos se ordenaron dentro de un mismo grupo, lo cual se confirmo por medio de un análisis jerárquico de clases y mostro un mayor parecido entre estos dos sistemas de cultivo. Además demostro el análisis de clases similitudes tanto entre cultivos a cielo abiero convencionales y orgánicos como entre cultivos de invernadero convencionales y orgánicos. Los suelos pertenecientes a pastizales y áreas de referencia mostraron fuertes discordancias con las otras áreas de cultivo, por lo tanto, se les ordeno respectivamente dentro de dos grupos independientes. Mientras que en los suelos de pastizales se identifico un aumento significativo en biomasa y actividad enzimática, lleva cualquier cambio en el uso del suelo a una disminución de la respiración de basales y del cociente metabólico en comparación con los suelos del área de referencia. Sin embargo, no se pudo detectar diferencias significativas entre las diferentes formas de cultivo, el cultivo a cielo abierto e invernadero.

Todos los grupos taxonómicos microbianos identificados a través del análisis de los PLAG mostraron una disminución de biomasa en comparación a los suelos de referencia a consecuencia del uso agrario. En los suelos provenientes de barbecho se encontro que la disminución de biomasa era menos significante, debido al ingreso de material orgánico en forma de excremento animal. Los suelos del campo mostraron un contenido más elevado de PLAG en comparación con los suelos situados bajo invernaderos. Además al comparar los suelos provenientes de cultivos convencionales con aquellos provenientes de cultivos orgánicos se encontro que en los orgánicos habia una mayor cantidad de bacterias Gram negativas y hongos. A parte no fue posible detectar ningún otro efecto dependiente de la forma de cultivo. Por lo general se identificaron en los suelos de barbecho el menor contenido en grupos taxonómicos lo cual muestra que los microorganismos del suelo necesitan de un largo periodo para recuperarse del uso agrícola. Al comparar los suelos del área sin influencia antrópogena con los suelos de cultivo se encontro que el porcentaje de los

siguientes grupos microbiales era mucho más bajo: actinomycetes, bacterias anaerobias Gram positivas y especialmente bacterias Gram positivas aerobias, bacterias anaerobias. Las actinomycetes presentaron solamente en los suelos de pastizales valores menores a los del suelo de referencia. Al comparar los suelos de todos los sistemas de cultivo con los suelos de referencia se encontro en todos los suelos un porcentaje más alto, a pesar de que su contenido total de PLAG se habia reducido, de protozoa, hongos y bacterias Gram negativas, a excepción de los suelos provenientes de cultivo convencional de invernadero y barbecho donde el porcentaje encontrado de protozoa fue menor al de los suelos de referencia. En suelos del campo y barbecho se encontro incluso una cantidad significantemente mayor de bacterias Gram negativas en comparación con aquellos suelos de otros sistemas de cultivo. No se detectaron diferencias entre los sistemas de cultivo o entre cultivo a campo abierto e invernadero. Un análisis jerárquico de clases reforzo los resultados esenciales obtenidos, mientras que a través de un análisis discriminante no fue posible catalogar los diferentes sistemas de uso del suelo.

Con el fin de poder evaluar en la investigación de los diferentes sistemas de uso del suelo las estructuras sociales microbianas del suelo y sus funciones, se análisaron los mismo parámetros descritos previamente en la valoración del experimento de campo. Tanto para los parámetros funcionales como estructurales se llevo a cabo una ACP. Para los parámetros estructurales se determinaron practicamente los mismos cuatro componentes principales capacidad microbiana, efectividad de mineralización, potencial de transformación de nitrógeno y actividad metabólica, pero no fue posible realizar una mejor clacificación para los PLAG análisados ya que la base de datos era demasiado homógena, las condiciones de clima y clima de suelo eran iguales y además se habia realizado hace poco un cambio en el tratamiento del suelo.

De nuevo se calculo un análisis discriminante para los parámetros funcionales, a partir de los valores factor de la ACP los cuales pudieron marcar una diferencia clara entre barbecho, cultivo convencional y orgánico, y parcelas sin tratamiento alguno. Sin embargo no fue posible identificar una diferencia entre los suelos pertenecientes al mismo método de cultivo y diferencias en la aplicación de pesticidas. Tampoco fue posible con relación a la cantidad aplicada de pesticida. A pesar de todo, es factible concluir que un cambio en el uso del suelo influencia la funcionabilidad del suelo y que determinar estos parámetros podría ser una

heramienta para evaluar la salud del suelo y la transformación de barbechos en campos cultivados convencionalmente o orgánicamente.

Los PLAG 14:0, 17:0 y 10Me17:0 al igual que los grupos taxonómicos microbianos de los protozoa, los cuales se encuentran representados por los PLAG 20:4ω6, reaccionan, tanto en porcentaje relativo como total, con la mayor sensibilidad hacia las diferentes palicaciones de fertilizantes. Al concluir un exprimento de aplicaciones fuertes se da una reducción mayor en el valor total de la suma de PLAG, la cual representa toda la biomasa, bajo las aplicaciones del tramiento orgánico a que bajo las aplicacionese del tratamiento convencional. Al igual que en las funciónes sociales microbianas del suelo, no se encontro ningúna reacción dentro de la estructura a las aplicaciones de pesticidas de ningúna clase, ni tampoco bajo ningúa cantidad. Además no fue posible detectar la aplicación de la Gram positiva *Bacillus thuringiensis* a través del anális de los PLAG. Sin embargo con la ayuda del análisis discriminante calculado a través de los valores factor de la ACP de los PLAG, fue posible marcar una diferencia clara entre los suelos del barbecho, cultivos orgánicos y convencionales y de las parcelas sin tratamiento alguno. Aunque no fue hasta despuése de la sexta semana de aplicación de fertilizantes que se identifico una diferencia entre los suelos de parcelas sin tratamiento y los suelos de parcelas del cultivos convencionales.

Finalmente se encontraron, en las dos partes de esta disertación, corelaciones significantes entre los contenidos absolutos de PLAG de cada grupo taxonómico microbiano y los parámetros funcionales microbianos del suelo, lo cual indica una estrecha relación entre estructuras sociales microbianas del suelo y sus funciones. Por lo tanto, la compoción social microbiana por si sola no conforma ningún valor indicativo y tiene que relacionarse con los parámetros funcionales. A través de la evaluación de parámetros individuales no fue posible determinar ni diferencias significantes entre suelos pertenecientes a diferentes sitemas de cultivo ni dentro del experimento de campo. Consecuentemente se puede concluir que estos Mollisoles arcillosos y limosos situados en la zona interna del cinturón verde que rodea a Buenos Aires, que muestran una disponibilidad biológica potencial reducida de xenobiotica, no reaccionan sensibles hacia el uso intensivo agrario. A pesar, de que nose encontro una degradación del suelo, ni a corto o mediano plazo a causa de este uso, como se mostro en el experimento de campo y en las investigaciones de los diferentes sistemas tipicos de cultivo, se recomienda fuertemente seguir estudiando los efectos que podrían darse a largo plazo.

Summary

In Buenos Aires, capital of Argentina, the fast growing population is causing a decrease in the agriculturally available area on the one hand and also a higher demand for food on the other hand. This mega-city is surrounded by an agricultural green belt with fertile Mollisols primarily providing the entire supply for the urban population especially with vegetables. In order to produce enough food, intensification of agriculture and maintenance of sustainable soil fertility are of essential importance. However, meanwhile the population is increasingly concerned about the negative aspects of this agricultural intensification being environmental contamination because of an enhanced application of pesticides and heavy metal containing fertilisers, eutrophication due to a higher manure addition and probably soil degradation in the course of which organic management is getting increasing attention. The inner zone of the green belt is agriculturally used in manifold ways. Besides fallows and pastures, conventional and organic cultivation are conducted on open fields and under greenhouses, while also anthropogenically unaffected areas with soils representing a reference for natural soil condition still exist. Various land use systems and diverse agricultural practices having the potential to change soil ecology and environment strongly influence soil microbial communities. Soil microorganisms are not only very important for fertility and sustainability of agricultural soils, they also play an essential role in global element cycles.

The objectives of this dissertation were to examine whether diverse agricultural practices with varying inputs of pesticides and heavy metals cause soil pollution and whether the different land use systems lead to an alteration of soil microbial community structure and function. Additionally, it was investigated whether conventional cultivation leads to a soil quality deterioration and if a replacement by organic cultivation can improve this situation. It was also tested whether land use conversion from fallow to typical land use systems of the investigation area influences soil microbial community structure and function with special emphasis on pesticide application and conventional as well as organic management. For this purpose, a monitoring on existing typical land use systems was conducted as well as a field experiment during a complete vegetation period of nine weeks was designed starting from a fallow area being tilled and exhibiting the natural variability besides non-tilled fallow plots. In this experiment conventional treatments were simulated using mineral fertilisers as well as both the insecticide endosulfan and the fungicide chlorothalonil alone or in combination,

while organic treatments were simulated using humus as well as both the biological insecticide *Bacillus thuringiensis* and the fungicide copper oxychloride also alone or in combination.

In top soils of the different land use systems except for the insecticides cypermethrin, deltamethrin, malathion and triazofos, which were below the detection limit (0.1 μg kg^{-1}), the insecticides carbofuran, chlorpyrifos, dimethoate, endosulfan and permethrin, the fungicide chlorothalonil and the herbicide trifluralin could be quantified between 0.2 and 34.2 μg kg^{-1} exclusively in conventionally managed soils of agricultural fields and greenhouses and in one fallow soil, which was previously also conventionally cultivated. In the soils of the remaining land use systems all of these pesticides were below the detection limit. The mean heavy metal contents (Fe 17.1 – 18.9 and Mn 0.60 – 0.79 g kg^{-1}; Cu 16.8 – 19.2, Ni 6.8 – 7.4, Pb 10.6 – 13.2 and Zn 32.5 – 39.8 mg kg^{-1}) did not differ significantly ($P < 0.05$) between the diverse land use systems. Cadmium was only detectable in one fallow (0.9 mg kg^{-1}). Thus, the contamination hazard due to pesticides and heavy metals in the green belt around Buenos Aires can be regarded as low. Consequently, no alarming risk potential for human health, soil and water quality, plant growth and wildlife from agriculture is expected.

For the characterisation of the soil microbial community structure and function in soils of the different land use systems twenty phospholipid fatty acids (PLFA) and the following parameters were examined, respectively: enzyme activities (acid phosphatase, arylsulfatase, cellulase, dehydrogenase and urease), basal and substrate-induced respiration, soil microbial biomass, metabolic quotient, net nitrogen mineralisation, net nitrification and potential denitrification. In order to reduce data and to combine correlating parameters two principal component analyses (PCA) were carried out, one for the structural and one for the functional parameters. For the first ones four principal components (microbial capacity, mineralisation activity, nitrogen transformation potential and metabolic activity) were extracted, while for the PLFA six microbial taxonomic groups (Gram positive, anaerobic and aerobic as well as Gram negative bacteria, actinomycetes, protozoa and fungi) could be differentiated.

With the aid of a subsequent discriminant analysis of the factor scores calculated by the PCA of the functional parameters six independent land use groups could be differentiated. Nearly all soils of conventionally managed greenhouses, organically managed greenhouses and agricultural fields, pastures and reference sites could be allocated to the expected land

use groups. A shift between indoor and outdoor cultivation in organic management systems was obvious. Soils of conventionally managed agricultural fields and fallows were combined into one group being confirmed by a hierarchical cluster analysis and exhibiting the highest similarities between the soils of these two land use systems. In addition, the cluster analysis demonstrated similarities between conventional and organic outdoor as well as between conventional and organic indoor cultivations. The reference and pasture soils formed two more own clusters in comparison to the remaining land use systems because they exhibited great differences compared to those of the agricultural managements. Any land use change led to a heavy reduction in basal respiration and metabolic quotient when compared to the reference soils, while the pasture soils were distinctly enhanced in microbial biomass and enzyme activities. However, no significant differences in soil microbial community functions were detected between the management systems and in- and outdoor cultivation.

In the course of agricultural land use all taxonomic groups of microorganisms acquired by the PLFA analysis showed a reduction in biomass relative to the reference soils. The lowest microbial biomass reduction was detected in pasture soils because of organic matter input through excrements of animal stocks, while soils of agricultural fields exhibited higher PLFA contents than those under greenhouse cultivation. Higher contents of Gram negative bacteria and fungi were determined in organically managed soils when compared to the conventional ones. Further effects of the management systems under study could not be identified. Fallow soils generally had the lowest contents of the taxonomic groups indicating that soil microbes need a long time to recover from agricultural land use. Compared to the anthropogenically unaffected soil, the relative abundances of microbial groups in soils of the other land use systems resulted in distinctly lower percentages for actinomycetes, Gram positive, anaerobic and particularly Gram positive, aerobic bacteria. Only in pasture soils the actinomycetes had higher values than the reference soil. Higher proportions were found for protozoa, fungi and Gram negative bacteria in soils of all land use systems – although they decreased in absolute PLFA contents – with the only exception of conventional greenhouse and pasture soils, which exhibited lower proportions for protozoa than the reference soils. Gram negative bacteria even had significantly higher values in agricultural field soils and fallows compared to those of the remaining land use systems. Differences in management systems or outdoor and indoor cultivation were not detected regarding the relative abundances of the taxonomic

groups. In addition, a differentiation of the land use systems by a discriminant analysis was not possible, while in general a hierarchical cluster analysis confirmed the results mentioned above.

For the examination of the field experiment the same parameters as described above were analysed in order to assess soil microbial community structure and function as described for the investigation of soils of the diverse land use systems. Resembling, a PCA was performed both for the functional and for the structural parameters whereas nearly the same four principal components were extracted for the functional ones namely microbial capacity, mineralisation activity, metabolic activity and nitrogen transformation potential. However, a reasonable classification of the analysed PLFA into specific microbial taxonomic groups was impossible caused by a too homogenous data set due to the same soil and climatic conditions and recent changes in treatments.

Again a subsequent discriminant analysis calculated by the factor score of the PCA with the functional parameters could clearly distinguish between soils of fallow, conventionally as well as organically managed and non-treated agricultural plots. However, a differentiation between soils of the same management with respect to different pesticide applications was equally impossible as a discrimination of varying application amounts. Nevertheless, it is concluded that land use conversion does influence soil functionality and determining soil microbial functions is a potential tool to monitor soil health and conversion of fallows into both conventionally and organically managed fields.

The most sensitive PLFA responding to different fertiliser applications were PLFA 14:0, 17:0 and 10Me17:0 as well as the taxonomic microbial group of protozoa being represented by PLFA 20:4ω6, which exhibited differences in both absolute and relative abundances. After strong fluctuations the total PLFA content representing the viable biomass showed slightly lower values in organic compared to conventional treatments at the end of the experiment. Similar to the soil microbial community function the pesticides did not cause any effect on the structure with respect to both kind and amount of application. Furthermore, the addition of the Gram positive *Bacillus thuringiensis* was not detectable by PLFA analysis. However, by means of a discriminant analysis computed with the factor scores of the PCA calculated with the PLFA profiles it was clearly possible to distinguish among soils of fallow, organically as well as conventionally managed and non-treated plots whereas those of the latter ones and

of the conventionally managed plots were not differentiable before six weeks after fertiliser application.

Finally, significant correlations between absolute PLFA contents of all analysed microbial taxonomic groups and soil microbial functional parameters were found in both parts of this dissertation indicating close connections between soil microbial community structure and function. Therefore, microbial community composition on its own is of no indicator value for soil quality and has to be combined with functional properties. Individual investigated parameters alone neither revealed significant differences between soils of the diverse land use systems nor within the field experiment. Consequently, it can be concluded that these silty and clayey Mollisols possessing a potentially low bioavailability to xenobiotics are not sensitive against intensive agricultural utilisation as in the green belt around Buenos Aires. Although it was revealed that soil degradation does not occur after this utilisation at the short- and medium-term as shown by the results of the field experiment and the monitoring on the typical land use systems, respectively, it is strongly recommended to further test these effects in the long term.

1. Introduction

1.1 Background

Buenos Aires, the capital of Argentina, is one of the ten most populated mega-cities in the world where about one third of the Argentinean population lives. There are about 14 million inhabitants in the metropolitan area and nearly 2.8 million in the city center. The annual population growth presently comes up to one per cent, which corresponds to an increase of about 140,000 people per year (Kobert *et al*., 2004). Since about 1990 many citizens have moved from the city into periurban areas. Many private quarters, so-called *Country Clubs*, have been established with the consequence of a dramatic reduction in areas usable for agriculture. Only a small green belt around this mega-city remained to supply people with vegetables. This green belt is subdivided into two zones of different degrees of urbanisation and land use intensity. The outer zone is characterised by a low population density but a higher degree of agricultural use because the urban expansion has not reached this region, yet. The situation of the inner zone including the administrative districts of the province of Buenos Aires close to the city centre is reverse. It shows a high population density and a comparatively small area dedicated to agriculture. However, the increasing demand of vegetables for the urban market must mainly be covered by this decreasing agricultural area of the inner zone of the green belt. From this situation a rising pressure results onto the local farming. Agricultural ecosystems are profit-oriented where productivity is more important than environmental issues. In those systems the net primary production has to be optimised through control mechanisms like nutrient supply and plant protection methods. In that way limiting factors of plant growth and development are eliminated for maximal exhaustion of production potentials. The maintenance of sustainable soil fertility in agriculture is of main interest because only thus the local food production of the fast growing population can be guaranteed. Hence, this situation can only be satisfied by enhanced use of improved seeds, pesticides and fertilisers in order to increase harvest yields. However, long-term effects on soil contamination are poorly studied in Argentina.

Over the past fifty years pesticides have been used increasingly in the environment. The ideal pesticide should only be toxic to the target organisms, biodegradable and should not leach into groundwater. Unfortunately, this is rarely the case and the widespread use of

pesticides in modern agriculture is of growing concern (Johnson *et al*., 2001). It has been estimated that less than 1 % of 2.5 million tons of pesticides applied annually is absorbed by the target organisms, while the rest is taken up by soil, water, air and non-target organisms (Andrade *et al*., 2005). Although pesticides are intended to protect crops, they may alter the equilibrium of soil processes by direct or indirect action on the soil microbes for shorter or longer periods depending on the intensity and spectrum of active agents and persistence of the parent chemical or its metabolites (Chen *et al*., 2001a). For example, in Argentina raised soybean production was closely followed by enhanced pesticide utilisation, which increased from 39 to 124 Gg between 1991 and 1997 (Jergentz *et al*., 2004). A comparable situation of massive pesticide application induced by the high number of harvests per year (up to five under greenhouses) is developed in regions with horticultural cultivation. Commonly used insecticides in soybean production around Buenos Aires are the pyrethroid cypermethrin, the organophosphate chlorpyrifos, the pyrethroid deltamethrin and the organochlorine endosulfan (Marino and Ronco, 2005). Pesticides are readily available for farmers but the cultivation practices performed by them are difficult to control (Jergentz *et al*., 2004). Various studies have already examined the contamination situation of organochlorine pesticides in rivers, surface and run-off waters (Rovedatti *et al*., 2001; Miglioranza *et al*., 2004a; Jergentz *et al*., 2005). Therein the pesticide contents are considered high but no evidence for potential hazard for wildlife could be found at present (Miglioranza *et al*., 2004b).

Heavy metals are another potential problem since they severely affect microbial growth, morphology and metabolism in soils through functional disturbance, protein denaturation or destruction of integrity of cell membranes (Kandeler *et al*., 2000). They are unintentionally supplied to agricultural soils with manure or pesticides. Application of organic residues like humus or dung compost as a source of organic matter is a common practice in Argentinean agriculture (Lavado *et al*., 2005; Torri *et al*., 2003) in order to improve soil physical, chemical and biochemical properties (Entry *et al*., 1997). However, the use of organic fertilisers such as sewage sludge and biosolids can lead to problems with respect to the accumulation of heavy metals in soils (Sloan *et al*., 1997). Also inorganic, phosphate-containing fertilisers produced from rock phosphates with varying contents of trace and minor elements being widely used in Argentina could lead to heavy metal accumulation in agricultural soils (de López-Camelo *et al*., 1997). When applied to soils, these elements may persist due to their long residence

time in soils and could be readily available for plants, especially in acid soils (Kpomblekou-A and Tabatabai, 1994). The use of any kind of fertilisers was restricted in Argentina some years ago but the accelerated technological change has produced a real boom in fertiliser application (de López-Camelo *et al*., 1997). In addition, heavy metal containing fungicides like copper oxychloride are especially applied in organic agriculture (du Plessis *et al*., 2005). Furthermore, human activity within the areas of La Plata and Buenos Aires City generates serious pollution of surface waters, sediments and soils due to direct industrial discharges. There are more than 300 actual sources of pollution associated with chemical industries, metallurgy, wood and paper mills plus non-treated urban sewage (Ronco *et al*., 2001). For trace metals such as cadmium, lead and zinc the high enrichment factors in the atmosphere of these areas indicate that anthropogenic inputs are more important than natural sources (Bilos *et al*., 2001). Pollution of rivers derives from farming and wastewater released from domestic sewage and thousands of industrial plants (Magdaleno *et al*., 2001). Consequently, besides pesticides there might additionally be an important heavy metal burden on the ecosystems around Buenos Aires with respect to all these potential sources with a possible hot spot in agriculture.

Two different agricultural management systems have developed intending to satisfy the present demand for vegetables. On the one hand, the conventional cultivation system is an industrialized agricultural system characterized by mechanisation, monocultures and the use of synthetic agrochemical inputs such as plant growth regulators, mineral fertilisers and pesticides with an emphasis on maximizing productivity and profitability. On the other hand, the organic cultivation system is a holistic production system, which promotes the role and health of agroecosystems by conservation of biodiversity, biological cycles and soil microbiological activity without the use of genetically modified seeds and plants or synthetic agrochemicals. Differences in tillage systems between these two cultivation types do not exist. Organic in contrast to conventional farming management relies on crop rotation, animal manure, crop residues and mechanical cultivation in order to keep soil productivity. Additionally, the development of microbiological diversity in the fields to disrupt habitat for pest organisms is as important as the purposeful maintenance and replenishment of soil fertility, as well as plant disease, pest and weed control (Petersen *et al*., 1997). The amounts and variety of organic inputs are much higher than in conventional agriculture. In addition,

the microflora of these soils play an important role in mediating the release of nutrients from crop residues, manure and soil organic matter (Petersen *et al*., 1997). These two management systems are practiced both on agricultural fields and under greenhouses within the study area. Cultivation under greenhouses enables an increase of harvest frequency from three to five per year. Further agricultural land use systems in this inner zone of the green belt around Buenos Aires comprise fallows as areas of soil recovery from intense cultivation and pastures. Additionally, one site still occurred, which was anthropogenically unaffected.

Variations in land use may strongly affect soil ecology and thus, they are likely to disturb soil microbial functions and changes in microbial community structure may in turn impact soil processes. Agriculturally used soils are sensitive to anthropogenic disturbances such as compaction and intensive utilisation, while alterations of agricultural use like management system, organic and mineral fertiliser application, pesticide input and cultivation practice influence quantity, dispersion and dynamics of soil microorganisms in an agroecosystem potentially leading to short, medium and long term alterations in soil productivity (Joergensen and Emmerling, 2006). Consequently, it is hypothesised that soil properties being fundamental for sustainability and productivity of soils have greatly suffered beneath intensive conventional management since plenty of synthetic agrochemicals have been applied to these agricultural fields. Joergensen and Emmerling (2006) reported that soil contamination with heavy metals or pesticides influence microbial groups and functions. Unlike, the more gently organically cultivated soils could rather have maintained higher activity and potential of physical, chemical, biological and biochemical parameters due to less input of xenobiotics into these agroecosystems. There is growing evidence that organic management systems positively influence soil quality characterised by higher biological activity in comparison to the conventionally managed soils (Emmerling, 2005; Monokrousos *et al*., 2006). On fallows, soil conditions should be less affected because of recovery during cultivation break whereas on pastures soil functions could be affected by soil compaction and excretal returns (Šimek *et al*., 2006). However, the site, which has not been under any cultivation or utilisation, reflects the natural state of a soil. Therefore, the comparison of soil properties of the Argentinean land use systems with those of a reference located in the same area should exemplify the changes of soil microbial community structure and function of soils due to land use.

1.2 Soil quality evaluation

Soil quality has been defined as the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries to sustain plant and animal growth, to maintain or enhance environmental quality and to support human health and habitation (Doran and Parkin, 1994; Karlen *et al*. 1997). Hence, appreciation and understanding of soil properties and ecology is increasingly recognised as important for the maintenance and sustainability of natural systems. In every ecosystem the composition and activity of soil microorganisms play important roles in biogeochemical cycles, degradation and recycling of organic residues and nutrient transformation and cycling leading to nutrient availability for plants. Turn-over and mineralisation rates of the main nutrient elements carbon, nitrogen, phosphorus and sulfur are mainly controlled by the microbial activity (Chander *et al*., 1999). Although soil is a dynamic system appearing to be in equilibrium, this equilibrium is precarious since any disturbance of the soil environment or any change of land use has the potential to modify microbial populations and thus soil fertility (Chen *et al*., 2001a). The evaluation of soil quality and sustainability is a crucial issue for which strong efforts have been attempted in the last years for definition and measurement (Puglisi *et al*., 2005; Joergensen and Emmerling, 2006).

On the one hand, it is assumed that soil microorganisms are more sensitive to human disturbance than soil chemical and physical properties (Degens *et al*., 2001) but on the other hand, they are able to accommodate to possible environmental constraints at the microbial level by adaptation and selection processes as well as by adjusting activity rates, biomass and community composition (Schloter *et al*. 2003a). However, still no generally accepted strategies exist for testing, surveying and evaluating the habitat function of soils for microbes (Kördel and Römbke, 2001; Johnsen *et al*., 2001). With respect to the selection of parameters as adequate soil quality evaluation indicators Doran and Parkin (1996) recommended a 'minimum data set' including physical (texture, rooting depth, infiltration rate, bulk density and water retention capacity), chemical (pH value, total carbon content, nutrient levels and electrical conductivity) and biological (microbial biomass carbon and nitrogen, potentially mineralisable nitrogen and soil respiration) properties. The traditional approach to study soil quality was the determination of basic soil properties such as total organic carbon, total organic nitrogen, pH value, cation exchange capacity and available nutrients (Glaser *et al*., 2001). In general, the physical and physico-chemical parameters are of little use as they alter

only when the soil undergoes a really drastic change (Filip, 2002). On the contrary, biological and biochemical parameters are sensitive to the slight modifications soils can succumb in the presence of any varying influencing factors (Yakovchenko *et al.*, 1996; Nannipieri *et al.*, 1990). Hence, for the assessment of the effects of different land use concerning soil sustainability, quality and natural properties, microbial variables constitute important criterions (Doran and Parkin, 1994; Franzluebbers *et al.*, 1995; Joergensen and Emmerling, 2006). Thereby main focus should rely on consideration of key soil indicators, which have to include both general and specific biological and biochemical parameters whereby conclusions about activities and potentials of soil microbes and about turn-over processes in nutrient cycles and energy flows could be derived (Gil-Sotres *et al.*, 2005).

In order to characterise differences of soil microbial community functions caused by the above-mentioned land use systems several biochemical parameters should be determined. Soil enzyme activities are suggested as suitable soil quality indicators because they are a measure of the soil microbial activity (Nannipieri *et al.*, 2002) and thus, they are strictly related to the nutrient cycles (Monreal and Bergstrom, 2000; Tabatabai and Dick, 2002). Soil enzymes are mostly generated by microorganisms and act either intra or extra cellular whereas many enzymes catalyse specific reactions for organic matter transformation in soil (Gianfreda *et al.*, 2005). Dehydrogenase and cellulase activities are strongly correlated with microbial biomass, while arylsulfatase, phosphatase and urease are important enzymes of cycles of the main nutrition elements sulfur, phosphorus and nitrogen (Chander *et al.*, 1999). Soil enzymes may rapidly respond to environmentally and anthropogenically induced stress (Gianfreda and Bollag, 1996) and are considered as early and sensitive indicators for the determination of the degree of soil degradation in both natural and agroecosystems (Dick, 1997). Therefore, they are well suited to characterise the impact of pollution (Trasar-Cepeda *et al.*, 2000) and cultivation on soil quality (Dick and Tabatabai, 1993). Although a significant correlation between microbial biomass and soil organic matter often was observed (Chander *et al.*, 1999), the microbial biomass is a labile pool that is more sensitive to all environmental disturbances than to other pools of soil organic matter (Gregorich *et al.*, 1994). Soil respiration is another parameter providing an index for both the microbial biomass and the microbial activity, while the metabolic quotient is considered as a sensitive indicator for soil microbial disturbances (Jones and Ananyeva, 2001). Generally, changes in soil microbial biomass,

metabolic quotient as well as basal and substrate-induced respiration rates are often accounted as early warnings of changes, which may occur in the long term with regard to soil fertility and agroecosystem properties (Emmerling and Udelhoven, 2002; Degens *et al.*, 2001; Kaiser *et al.*, 1995; Teklay *et al.*, 2006). Cultivation practices, which are associated with intensification of agriculture are well known to alter soil microbial biomass and activity (Emmerling *et al.*, 2001; Gianfreda *et al.*, 2005; Wardle *et al.*, 1999). All main components of the nitrogen cycle such as nitrification, denitrification and nitrogen fixation also depend on accordant microorganisms (Pell *et al.*, 1998). Denitrification serves as an indicator of land use variations since it depends on soil properties and land use practice. Pasture soils for example emit more nitrous oxide when compared to agricultural soils caused by urine entry and compaction (van Groeningen *et al.*, 2005). Furthermore, agricultural fields receiving organic fertilisers particularly in organic managements show higher nitrous oxide emissions than grasslands (Mogge *et al.*, 1999). Soil cultivation and residue quality have a significant impact on nitrogen cycling and nutrient contents (Raiesi, 2006). Therefore, nitrogen mineralisation is another adequate soil microbial indicator for determining the alteration of soil properties caused by changing land use or agricultural intensification. Many pesticides have the ability to eradicate parts of the soil biology or to influence the numbers and community of a diverse range of soil microorganisms that contribute to soil biological processes and maintain soil structure and fertility (Chen *et al.*, 2001a). Moreover, management practices, particularly the input of fertilisers and pesticides, can have large impact on size and activity of soil microbial communities (Bossio *et al.*, 1998). Furthermore, there is a growing evidence suggesting that microbes are far more sensitive to heavy metal stress than plants and animals inhabiting polluted soils (Ekelund *et al.*, 2003).

Recently, soil microbial community structure has also been recommended as a biological indicator of soil quality, although there are various ways to quantify this structure e. g. DNA fingerprinting and denaturating gradient gel electrophoresis (DGGE, Ibekwe *et al.*, 2002), the determination of amino sugars (Glaser *et al.*, 2004) and phospholipid ether lipids (PLEL, Gattinger *et al.*, 2002) or community level physiological profiling (CLPP, Yao *et al.*, 2000). Several studies indicated phospholipid fatty acid (PLFA) analysis as a powerful and sensitive tool for soil status evaluation. PLFA are essential membrane components of all viable cells, which are not found in storage products or in dead cells (Zelles, 1999). Thus, by using the

composition of PLFA it is ensured that measurements will be on the living part of the microbes, since PLFA are considered to decompose quickly when organisms die. Therefore, PLFA pattern can be viewed as an integrated measurement of all living soil microorganisms reflecting both species composition and relative abundance. The use of PLFA profiles is based on the fact that microbes contain a relatively constant proportion of their biomass as phospholipids (Ibekwe and Kennedy, 1998). Therefore, identification and quantification of individual PLFA permit the detection of changes in microbial populations, since the latter stand for the present microbial communities in the soil (Ibekwe, 2004). Moreover, the total PLFA content serves as an index of the viable microbial biomass (Calderon *et al*., 2000). A possible change in microbial biomass can occur either by proportional growth of a microbial population without major changes in community composition or by different growth of microbial populations resulting in a shift in community composition (Steinberger *et al*., 1999). The PLFA pattern of living microbial cell membranes provides an insight into the diversity of microbial community. In practice, the PLFA are extracted from soil samples, purified and separated by chromatography in order to obtain the PLFA pattern of the sample. While PLFA profiles do not reveal species-level information directly, the lipid analysis provides a fingerprint of microbial diversity present at the time of sampling (Wander *et al*., 1995). Multivariate analysis of these profiles can be used to investigate compositional changes of soil microbial communities caused by alterations of environmental conditions. It is also possible to examine specific PLFA within the profile as molecular markers of different taxonomic microbial groups such as Gram positive or Gram negative bacteria, protozoa, actinomycetes and fungi (Zelles, 1999). Changes in PLFA patterns of microbial communities in soils subjected to a wide range of ecological conditions and alteration events would indicate a variation in microbial composition, which has been used to compare different land use systems (Bossio *et al*., 2005 and 2006) or various stress conditions (Allison *et al*., 2005). Land use is a strong determinant of soil microbial community and biomass (Waldrop *et al*., 2000; Burke *et al*., 2003). Previous studies exhibited that grassland and agricultural soils support distinct microbial communities that are correlated with factors, which define soil quality, suggesting that land use affect microbial community composition (Steenwerth *et al*., 2002 and 2005). The environmental conditions to which a soil has been exposed may also affect soil microbial biomass and community composition (Steinberger *et al*. 1999). In some

cases cultivation history had long-term effects on soil microbial community structure in agricultural fields (Ibekwe and Kennedy, 1998 and 1999), and gradients in soil fertility in either grasslands (Steenwerth *et al*., 2002) or cultivated sites (Yao *et al*., 2000) have been shown to influence microbial community composition. Agricultural management practice such as inputs of manure, cover crops, mineral fertilisers and pesticides in different amounts can have large impacts on the size, activity and composition of soil microbial communities (Bossio *et al*., 1998; Bossio and Scow, 1998), while changes in PLFA compositions can be distinguished following specific management practices over a cropping season (Bossio *et al*. 1998; Bai *et al*. 2000). Alike, residue incorporation, cropping sequence, irrigation and tillage alter soil microbial biomass (Franzluebbers *et al*., 1995; Ibekwe *et al*., 2002) and community structure (Lundquist *et al*., 1999; Calderón *et al*., 2000 and 2001; Steenwerth *et al*., 2005). It has been demonstrated that distinct changes in specific microbial populations can occur even if community size, turn-over rates and activities seemed to be unaffected by pesticide addition (Johnsen *et al*., 2001). Some microbial groups may be suppressed due to the toxicity of the pesticide, while others may proliferate in arising ecological niches because they are able to use an applied pesticide as a source of energy or nutrients. Consequently, this may lead to successions in soil microbial community structure and thus, to altered activities at a later point in time. On this account, it has been suggested that measures of microbial community structure and function may be more sensitive to disturbance than assays focusing on general microbial processes or community size (Kelly *et al*., 2003; Schloter *et al*., 2003a). Therefore, biochemical parameters may be useful as highly sensitive bioindicators of disturbance and of the progress of remediation also since heavy metal impact on microbes may include changes in community structure and decreases in microbial biomass (Kandeler *et al*., 1996 and 2000).

1.3 Objectives

The population increase leading to a reduction of the periurban area around Buenos Aires available for agriculture enhanced the pressure on horticultural production of vegetables, which thus had to be increased. Hence, due to intensive agricultural practice and the rising input of agrochemicals eutrophication, soil contamination and degradation are expected in this area and thus, an evaluation of sustainability of typical agroecosystems is necessary in order to estimate the anthropogenic impact on agricultural soils (Vilglizzo *et al*., 2006).

One objective of this dissertation was to quantify the heavy metal and pesticide contamination in soils of actual land use systems in the green belt around Buenos Aires. In addition to the reference soil, which was not affected by man, topsoils of the following six land use systems were investigated in detail: Conventional and organic cultivation under greenhouses as well as on agricultural fields, fallows and pastures. As already mentioned, some studies have been carried out on environmental contamination in the investigation area. However, to date, only marginal information has been given about the contamination situation in Argentina in soils and especially in agriculturally used ones. Because of crop production intensification it is hypothesised that heavy metal and pesticide contamination is high in soils of this region. Hence, this investigation shall provide an overlook about the actual contamination situation in the area around Buenos Aires and should give important information for policy makers and stakeholders as a regulatory tool for further agricultural guidelines in the study area. Furthermore, an evaluation of a set of thirteen variables derived from ten test methods of soil microbial community functions for its ability to discriminate between the different land use systems and to check whether intensive agricultural land use deteriorates soil microbial functions was performed. For this purpose, activities of several enzymes related to the cycles of the main biologically important nutrients carbon, nitrogen, phosphorus and sulfur, the respiratory and the metabolic activity as well as fundamental nitrogen mineralisation processes were measured. Thus, a test procedure is suggested to distinguish between the different land use systems via multivariate statistics. A principal component analysis of the microbial functional parameters was used to reduce data and a subsequent discriminant analysis was conducted in order to determine their ability of differentiation. Finally, a hierarchical cluster analysis was carried out to exhibit similarities between soils of individual land use systems. Alike, the same statistical tools were used to characterise the influence of various agricultural land use on microbial community structure in soils of the inner zone of the green belt around Buenos Aires compared to the native site. Ibekwe and Kennedy (1999) constituted PLFA profiles as a tool to investigate community structure in agricultural soils. Special emphasis was put on an ecological interpretation of microbial groups after statistical evaluation of PLFA profiles and correlations with microbial functional parameters.

In Argentina investigations about the effects of agricultural land use on microbial community structure and function in soils regarding pesticide and fertiliser application have not been performed, yet. Moreover, impacts of agrochemicals on soil microbial populations and community level interactions are poorly understood. Thus, another aim of this work was to acquire adequate structural and functional soil quality indicators since soil microbiological parameters react very fast to changes of soil fertility caused by disturbances or overuse. Therefore, in a field experiment starting from a fallow, agricultural land use systems typical for the investigation area were developed temporally. On the one hand, organic cultivation was simulated by fertilising soil with humus and treating it alternatively with the biological insecticide *Bacillus thuringiensis* and the fungicide copper oxychloride, while on the other hand, conventional cultivation was simulated by adding mineral fertilisers and applying the insecticide endosulfan and the fungicide chlorothalonil. The effects of the pesticides alone and in combination on soil microbial community structural and functional parameters were examined. A field experiment was performed because interactions between pesticides and the soil microbiology under actual conditions in the agricultural field may differ from those in standard laboratory experiments (Beulke and Malkomes, 2001). Moreover, the advantage of an outdoor experiment consists in the study of temporal progressions of soil microbial parameters under natural field conditions. Therefore, the objectives were to demonstrate under equal climatic conditions and soil type whether conventionally and organically managed soils are accompanied by varieties in microbial biomass as well as in community structure and function and whether there are interactions between common application doses of commercially available pesticides or fertilisers and soil microbiology. From the results the duration of soil regeneration phases as well as analytical tools for the control of different fertiliser and pesticide use in conventional and organic farming can be deduced. A vegetation cover was established in order to get as natural conditions as possible because the rhizospheres of plants can play major roles in affecting the composition of the soil microbial community (Chen *et al*., 2001a). Again the same soil microbial community structural and functional parameters as mentioned above were analysed and statistically evaluated in the same way as those for the investigation of the soils of the different land use systems.

2. Materials and methods

2.1 Site description

Both studies were performed in the periurban area of Buenos Aires (34° 35' S, 58° 29' W), as part of the pampean area of Argentina in South America. Buenos Aires is located about 25 m above sea level at the Río de la Plata, a funnel-shaped estuary of the two rivers Río Paraná and Río Uruguay at the South American Atlantic east coast. The mean annual precipitation in this humid Pampa region is 1027 mm with the highest monthly amount in March (142 mm) and the lowest in June (61 mm). The annual temperature averages 17 °C with the highest in January (24 °C) and the lowest in July (5 °C). During the sampling period of the land use systems in May 2004, mean temperature was 12 °C and rainfall amounted up to 70 mm, which is typical for this month.

The periphery of Buenos Aires is dominated by agricultural structures where grazing management is conducted and predominantly vegetables and flowers are cultivated with no-tillage technique. In this periurban area two sites of the inner zone of the green belt around Buenos Aires were chosen for soil sampling: La Plata in the south-eastern and Pilar in the north-western part of Buenos Aires. At these sites six different agricultural land use systems were identified: pastures, fallows and conventionally as well as organically managed fields and greenhouses. All land use systems were long-term practices over many years except the fallow sites (at least two years), which have previously been conventionally cultivated. Additionally, one site was found, which has not been in agricultural or any other use for at least twenty years. This site was considered as a reference in order to reflect the microbial community structure and function of anthropogenically unaffected soils in order to estimate effects of agricultural land use. The soils under study were characterised by mollic A- and argillic B-horizons and thus were classified as Typic Argiudolls mainly derived from younger quaternary eolian deposits. Toward the east of the Pampa, soils were more clayey and transitions to Vertic Argiudolls and Vertisols occurred (Lavado *et al.*, 2004).

The area of the field experiment was located in Castelar likewise in the periurban area of Buenos Aires. During the experiment period from September to December 2004 the mean temperature was 17 °C and the rainfall amounted to 141 mm. The soil of the sample area was a Vertic Argiudoll as described by Morrás *et al*. (1998) for profile 1.

2.2 Field experiment

2.2.1 Experiment design

200 m² of a fallow area were plowed and flattened before 28 plots (1.5 m × 1.5 m) and three additional ones on the remaining fallow were established (Figure 1). After one week of equilibration and two weeks before the beginning of the experiment on all field plots wild oat (*Avena sativa*) was sowed (~ 200 g of seeds per plot) in order to obtain a homogenous vegetation cover. On one plot natural vegetation growth was compared with crop growth on the field plots. In addition to three reference field plots three agricultural land use systems (fallow, conventional and organic management) were simulated in diverse variations on nine plots each in threefold replication (Figure 1) as described below.

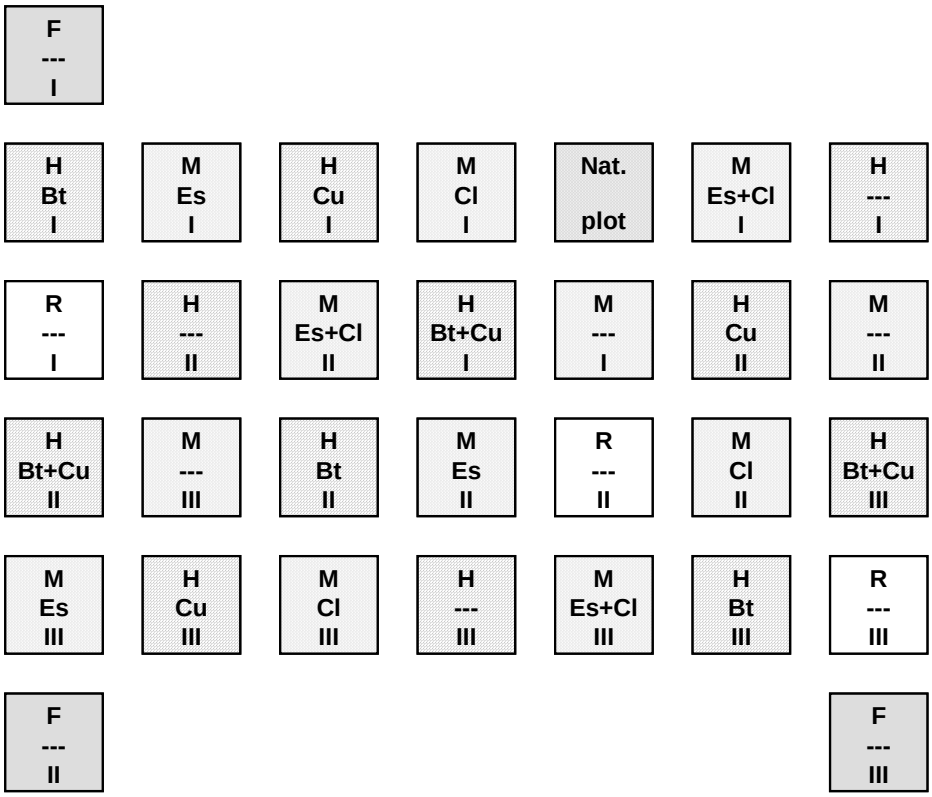

Figure 1: Plot layout of the field experiment; Nat. plot: natural plot for the comparison of natural vegetation and crop growth, F: fallow, R: reference field, M: mineral fertiliser, Es: Endosulfan, Cl: Chlorothalonil, H: humus, Bt: *Bacillus thuringiensis*, Cu: Copper oxychloride; I, II, III: plot replicates.

Fallow (i), conventional cultivation with mineral fertilisers (ii) plus endosulfan (iii), plus chlorothalonil (iv) or plus both (v), as well as organic cultivation with organic fertiliser (vi),

plus *Bacillus thuringiensis* (vii), plus copper oxychloride (viii) or plus both (ix), and reference field (x) without any fertiliser and plant protection procedure.

All conventionally managed plots were fertilised with 27.8 g of diammoniumsulfate and 95.8 g of diammoniumhydrogenphosphate (~ 116 kg nitrogen ha^{-1}, ~ 100 kg phosphorus ha^{-1}, ~ 30 kg sulfur ha^{-1}) at the day of oat sowing, one week after plowing and two weeks before pesticide application. To the plots with pesticide application 173 mg endosulfan (~ 770 g ha^{-1}, THIONEX-L, insecticide) or 405 mg chlorothalonil (~ 1,800 g ha^{-1}, ISATHALONIL 50 FW, fungicide) or a mixture of both were added three weeks after plowing (Figure 2).

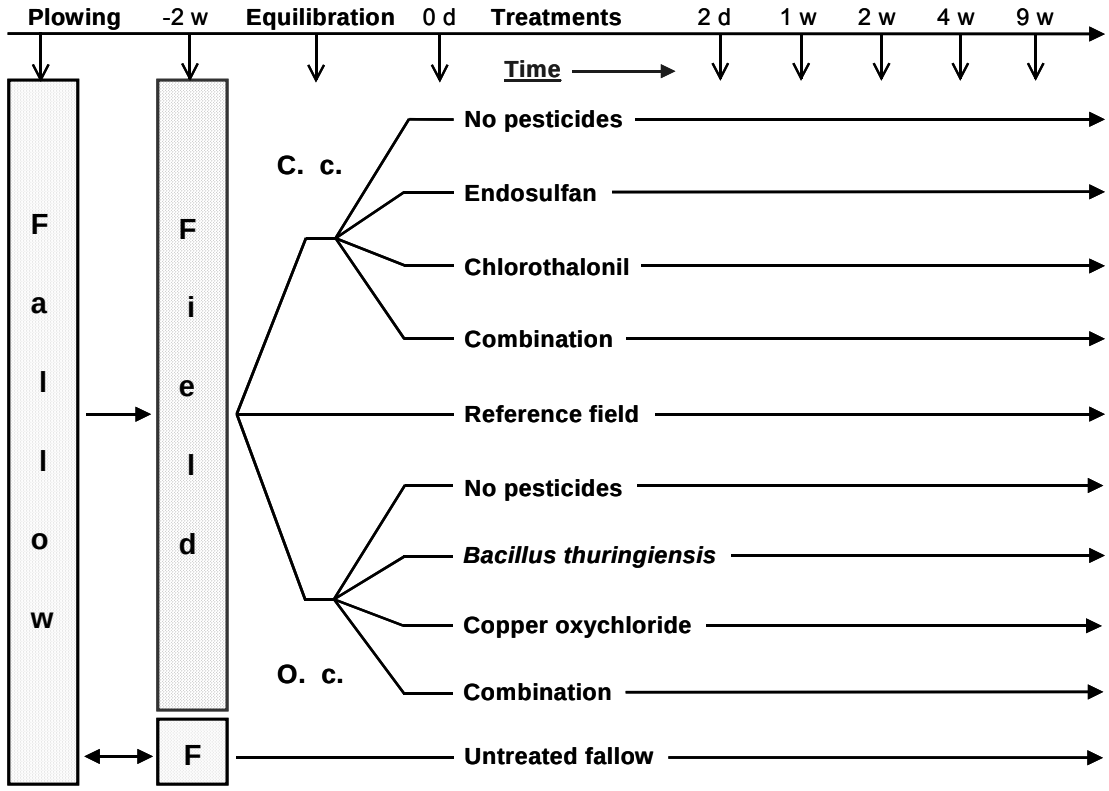

Figure 2: Chronological design of the field experiment; d: days; w: week(s); C. c.: conventional cultivation, O. c.: organic cultivation, F: fallow.

All organically managed plots were fertilised with 6 kg of humus (~ 26.7 Mg humus ha^{-1}; ~ 2.5 Mg TOC ha^{-1}, ~ 0.25 Mg TON ha^{-1}, ~ 37 kg S ha^{-1}, ~ 157 kg P ha^{-1}, pH 6.4) at the day of oat sowing, one week after plowing and two weeks before pesticide application. To the plots with pesticide application 16.9 mg *Bacillus thuringiensis* (~ 75 g ha^{-1}, BAC THUR, insecticide) or 371 mg copper (Cu) in 624 mg copper oxychloride (~ 1.6 kg Cu ha^{-1}, SUPERCUPROL, fungicide) or a mixture of both were added three weeks after plowing (Figure 2).

2.2.2 Pesticides

2.2.2.1 Endosulfan

The chlorinated sulfite diester endosulfan is a cyclodiene insecticide possessing a relatively broad spectrum of activity. Technical grade endosulfan is a mixture of the two stereoisomers α- and β-endosulfan in a ratio of 7:3 (Awashti *et al*., 1999). Endosulfan is used extensively throughout the world as a contact and stomach insecticide and as an arcaricide on field crops. Endosulfan does not dissolve easily in water, but sticks to particles or soil. It breaks down slowly and may accumulate in the environment and in organisms that are exposed to it (Antonious and Beyers, 1997). Because of its abundant usage and potential transport, endosulfan contamination is frequently found in the environment at considerable distances from the point of its original applications. Endosulfan has been detected in the atmosphere, soils, sediments, surface and rain waters and food stuffs and it is a priority pollutant for international environmental agencies. It is extremely toxic to fish and aquatic invertebrates and has been implicated in mammalian gonadal toxicity, genotoxicity and neurotoxicity (Siddique *et al*., 2003). Although several metabolites of endosulfan have been demonstrated to occur, only endosulfan sulfate exhibiting similar physico-chemical and toxic properties like endosulfan is significant as a residue (Antonious and Beyers, 1997).

2.2.2.2 Chlorothalonil

The organochlorine phthalonitrile chlorothalonil is a widely used foliar pesticide for the control of many fungal diseases in agricultural systems, especially in greenhouse production of fruits and vegetables (Yu *et al*., 2006). Chlorothalonil is a non-systemic fungicide with a wide coverage of action against a broad range of plant pathogens. It is used extensively worldwide and its residues have been detected in many vegetables (van Doorn *et al*., 1995). Several degradation pathways including substitution and conversion reactions have been identified providing manifold metabolites (Putnam *et al*., 2003). Nevertheless, the major degradation product in soil and plants is 4-hydroxychlorothalonil, which is acutely more toxic than the parent compound (Armbrust, 2001). The solubility of chlorothalonil in water is low why it is mainly absorbed in the top layer of soil (Kwon and Armbrust, 2006). However, its metabolite is easily soluble in water and thus the potential downward movement of this

compound is high if the adsorption capacity of the soil is low (van Doorn *et al*., 1995). Chlorothalonil is very toxic to fish and other water inhabiting animals and it is classified as a probable human carcinogen by the U.S. Environmental Protection Agency (Kwon and Armbrust, 2006).

2.2.2.3 *Bacillus thuringiensis*

Bacillus thuringiensis is a naturally occurring Gram positive bacterium common in soils throughout the world (Grove *et al*., 2001). Several strains can infect and kill insects and due to this property, *Bacillus thuringiensis* has been developed for insect control wherefore at present it is the only biological insecticide in widespread use. Unlike typical nerve-poison insecticides, *Bacillus thuringiensis* acts by producing proteins (δ-endotoxin, the toxic crystal) that react with the cells of the gut lining of susceptible insects. These proteins bind specific membrane receptors on the insect midgut brush-border epithelium, paralyse the digestive system and the infected insect stops feeding within hours. Affected insects generally die from starvation caused by intestinal cell lysis or from septicemia (Prieto-Samsónov *et al*., 1997). The proteins are selectively toxic to different species from several invertebrate phyla such as arthropods (mainly insects), nematodes, flatworms and protozoa. Some formulations can be used on essentially all food crops and *Bacillus thuringiensis* is considered safe to people and nontarget species such as wildlife (Grove *et al*., 2001).

2.2.2.4 Copper oxychloride

Copper is an essential trace metal for many organisms. However, at higher concentrations it is also a potentially toxic metal that can exert an inhibitory effect, which is the basis for formulation of copper-containing fungicides (Gharieb, 2002). Despite the production of a wide variety of synthetic organic fungicides, copper fungicides still predominate the field of fungicidal plant disease control. The copper fungicides have been used for the control of many vegetable, fruit and flowering plant diseases. The toxicity of copper compounds is due to their ability to precipitate proteins, which causes the coagulation of the cytoplasm. Copper oxychloride is probably the most widely employed copper fungicide. It has great efficacy against a wide variety of plant pathogenic fungi and also controls the pathogenic and non-pathogenic fungi associated with seeds. It may be applied alone, in combination with other

fungicides, seed treatment or foliar application. Copper fungicide formulations are poorly soluble in water and its fungicidal activity depends mainly on its solubilisation and availability of copper ions (Gharieb *et al*., 2004).

2.2.3 Fertilisers

2.2.3.1 Mineral fertiliser

Ammonium sulfate and diammonium hydrogen phosphate are used largely as artificial manure for alkaline soils and also for the preparation of other ammonium salts. In the soil sulfate and phosphate ions are released and form sulfuric and phosphoric acid, respectively, which lower the pH balance of the soil. Likewise these mineral fertilisers serve as high sources of essential nitrogen and contribute sulfur and phosphorus in plant available form for assimilation and plant growth. Ammonium sulfate is also used as an agricultural spray adjuvant for water soluble insecticides, herbicides and fungicides. There it functions to bind iron and calcium cations that are present in both water and plant cells. Ammonium sulfate, occurs in nature as the mineral mascagnite and both salts are soluble in water and insoluble in alcohol or liquid ammonia.

2.2.3.2 Organic fertiliser (humus)

Vermicompost (also called worm compost, worm humus or worm manure) is the end-product of the breakdown of organic matter by some species of earthworm. This humus is a nutrient-rich, natural fertiliser and soil conditioner. The process of producing vermicompost is called vermicomposting. For the present field experiment 60 % of horse manure, 20 % of herbage and 20 % of corn grist were mixed and moistened to 70 % of water holding capacity. This compost-water-mixture was incubated in so-called compost beds. Due to chemical and microbiological degradation processes the temperature inside the beds rise up to 70 °C so that the beds have to be turned over and aerated regularly. After the temperature inside the beds does not vary from that outside, the processes were completed. Thereafter red earthworms (*Eisenia fuetida*) were added to the compost passing it through their body and transforming it into humus rich vermicompost, which finally was applied to soil.

2.3 Sample collection

2.3.1 Land use systems

Surface soil samples were taken in 0 to 10 cm depth in at least 50 m distance on sites greater than 1 ha. The samples were sieved (< 2 mm), homogenised and immediately stored at –20 °C. Prior to the analysis the samples were carefully thawed in a refrigerator at +8 °C in order to prevent reactivation of microorganisms and to minimise pesticide losses through evaporation and mineralisation during soil sample storage. Every land use system was sampled in fourfold replication with the exception of pastures and organic greenhouse fields, which were sampled only in threefold replication because no more comparable sites were found with the same land use within the sampling area. For reasons of comparability only agricultural fields with cultivation of vegetables – preferentially lettuce – were sampled. In order to check infield homogeneity every site was sampled in threefold replication. The reference site was also sampled in threefold replication in at least 100 m distance between the three sampling points wherefore they were considered as independent sites.

2.3.2 Field experiment

Surface soil samples were taken on the fallow and the agricultural field two and one weeks before pesticide application. At the day, before the application, samples were taken on the fallow, the reference as well as on the organically and conventionally fertilised plots. Two days, one, two, four and nine weeks after pesticide application, samples were taken in every plot of the ten varying treatments (Figure 1). Every sampling was conducted in 0 to 10 cm depth using a quadratic sampling raster (1.5 m × 1.5 m divided into 25 squares each 30 cm × 30 cm in size). Fourfold sampling replication was done in every plot, one per raster square. The soil samples were combined, sieved (< 2 mm), homogenised and immediately stored at –25 °C. Prior to analysis the samples were carefully thawed in a refrigerator at +8 °C in order to prevent reactivation of microbes and to minimise pesticide losses through evaporation and mineralisation during soil sample storage.

2.4 Chemical analysis

2.4.1 Basic soil parameters

2.4.1.1 Texture

20 g of air-dried, sieved (< 2 mm) soil was mixed with 100 mL of distilled water and 5 mL of hydrogen peroxide and boiled at 90 °C for 4 h in order to destroy humic matter. After centrifugation at 3000 rpm for 15 min the supernatants were removed. The remaining soil was dried, weighed and represented 100 % for the calculation of the percentages of each fraction. For soil dispergation, 10 mL of 5 % sodiumhexametaphosphate solution were added to the samples, which then were shaken end-over-end for 2 h. The sand fractions were wetly sieved, dried and weighed. The filtrates were collected in 1 L cylinders, which were filled up with distilled water and allowed to equilibrate at room temperature. Thereafter the samples were shaken and by means of a pipette apparatus after Köhn silt and clay fractions were sucked off after sedimentation time required at given room temperature. All fractions were dried, weighed and the percentages of dry soil were calculated.

2.4.1.2 Water content

The percentage of the gravimetric loss of water from a 5 g field-moist soil sample was determined after an incubation period of 48 h at 105 °C.

2.4.1.3 pH value

10 g of air-dried soil were merged with 25 ml of distilled water and shaken end-over-end for 1 h. Then the samples were allowed to equilibrate at room temperature for 1 h. Thereafter the pH values were determined in the suspension of the soil samples.

2.4.1.4 Carbon and nitrogen contents

Approximately 30 mg finely ground soil and an equal amount of the initial weight of wolfram oxide were weighed into small tin boats. The boats were closed airtight and formed to small pellets. The pellets were put into the autosampler and analysed for carbon and nitrogen by an elementar Vario EL CN-analyser.

2.4.2 Pollutants

2.4.2.1 Pesticides

The following eleven pesticides were analysed according to the slightly modified method of Laabs *et al.* (1999): the insecticides carbofuran, chlorpyrifos, cypermethrin, deltamethrin, dimethoate, endosulfan (α- and β-isomer and its metabolite endosulfan sulfate), malathion, permethrin and triazofos, the herbicide trifluralin and the fungicide chlorothalonil.

All organic solvents (residue- or HPLC-grade) were purchased from Promochem, Wesel, Germany and deionised water was used (> 18 MΩ). Salts purchased from Merck, Darmstadt, Germany, were *pro analysis*-grade. Pesticide standards (purity > 97 %) were purchased from the Institute of Organic Industrial Chemistry, Warsaw, Poland, and Dr. Ehrensdorfer GmbH, Augsburg, Germany. All glassware was rinsed with technical acetone and ethylacetate, washed at 95 °C with a detergent and heated at 300 °C overnight before use.

25 g of field-moist soil and 50 mL of a solvent mixture of acetone/ethylacetate/water 2/2/1 (v/v/v) were merged in glass centrifuge vials and closed with teflon-lined screw caps. After vortexing, the vials were shaken end-over-end at 50 rpm for 4 h and centrifuged at 4000 rpm for 10 min. Internal standards (0.5 µg α-hexachlorocyclohexane (HCH), 0.5 µg terbuthylazine and 0.5 µg ditalimfos in toluene) were added to the supernatants, which then were decanted and filtrated through folded filters (Schleicher & Schuell, 596 ½). After adding a few drops of toluene, the filtrates were concentrated using a rotary evaporator (40 °C, 250 mbar). The remaining extracts were transferred into separating funnels and liquid-liquid extracted. For this purpose 25 mL of dichloromethane and – to enforce the transfer of the pesticides into the organic solvent phase – 5 mL of a saturated aqueous potassium chloride solution adjusted to pH 1 with hydrochloric acid (32 %) were added. After shaking the funnels on a horizontal shaker at 225 rpm for 10 min the lower dichloromethane phase was dried over anhydrous sodium sulfate. These separation steps were repeated twice, the three dried dichloromethane phases were combined and concentrated on a rotary evaporator (40 °C, 700 mbar) up to the toluene phase. Finally, a recovery standard (0.2 µg naphtalene-D_8 in toluene) was added to determine the recovery of the internal standards.

The samples were transferred into autosampler vials and analysed by a gas chromatograph (GC) connected to a mass selective detector (MSD) (GC: Hewlett Packard (HP) Series II 6890; MSD: HP 6971 A) and an autosampler (HP 7673). The pesticides were separated on a 30 m capillary column (HP-5 MS consisting of 5 % phenyl-methyl siloxane) with a film thickness of 0.25 µm and an inner diameter of 0.25 mm. Helium (purity: 99.996 %) was used as carrier gas with a column pressure of 48 kPa and 1 µL of sample solution was injected splitless (splitless time: 1.25 min). The injector temperature was 150 °C and the detector temperature was 310 °C. The temperature program was run as follows: Initial temperature 82 °C held for 2.5 min, increased at 10 °C min^{-1} to 130 °C, increased at 4 °C min^{-1} to 160 °C held for 10 min and finally increased at 10 °C min^{-1} to 280 °C held for 10 min. The ionisation was conducted by electron impact (70 eV) mode and the measurement was done in selected ion monitoring mode. The identification of pesticides was based on comparison with retention times and mass spectra, which were obtained from standards (Table 1). The internal standards were used to compensate sample processing losses, while the recovery standard was used to countervail apparatus-induced measurement variations and allowed to quantify the recoveries of the internal standards. The pesticide concentrations were calculated as outlined by Laabs *et al.* (1999). For analytes showing two or more peaks in the chromatogramm sum of peak areas (carbofuran I and II, permethrin I, II and III and cypermethrin I and II) were used for the quantification. Those internal standards used for quantification of particular pesticides are given above the accordant pesticides in the order of retention times as shown in Table 1. The mentioned target ions are specific fragment ions per analyte with the highest abundance, while qualifier ions are two further characteristic ions after ionisation in the mass selective detector (Table 1).

Besides chlorothalonil, endosulfan and its metabolite endosulfan sulfate the samples of the field experiment were additionally analysed for 4-hydroxychlorothalonil, the metabolite of chlorothalonil. The performance of the analysis was analogous to that described above. However, after the clean up, the samples had to be derivatised, which was done according to the slightly modified method of van Doorn *et al*. (1995). For this purpose, the samples were transferred into 4 mL reaction vials and after 2 mL of diazomethane were added the mixture was allowed to stay at room temperature for 15 min. After evaporating excess diazomethane by using a gentle stream of nitrogen, 3 mL of hexane were added and the solution was

concentrated to 1 mL, which was repeated twice. 2 g of 10 % deactivated aluminium oxide (MP Alumina N, activity I, *reinst*-grade, 50–200 μm, MP Biochemicals, Eschwege, Germany) was slowly poured into a chromatographic column, which was filled with hexane. The hexane was drained until the level reached the top of the aluminium oxide and then the hexane extract was transferred onto the aluminium oxide. The column was eluted with 5 mL of hexane, which were abolished and with 15 mL of 4 % ethyl acetate in hexane (v/v). The eluates were concentrated to 200 μL on a rotary evaporator (40 °C, 400 mbar) and a recovery standard (0.2 μg fluorene-D_{10} in toluene) was added to determine the recovery of the internal standard. The samples were transferred into autosampler vials with toluene and measured as described above for the other pesticides.

Table 1: Molecular weights, ion masses and retention times of pesticides and standards used for quantification and identification in selected ion monitoring mode.

Pesticides	Molecular weights [g]	Target ions [m/z]	Qualifier ions 1 [m/z]	Qualifier ions 2 [m/z]	Retention times [min]
α-HCH *	290.8	181	183	111	19.3
Chlorothalonil	265.9	266	264	268	24.9
α-Endosulfan	406.9	237	239	241	32.5
β-Endosulfan	406.9	237	193	243	34.1
Endosulfan sulfate	422.9	272	237	387	35.1
Ditalimfos *	299.3	130	299	243	32.6
Dimethoate	229.2	93	125	63	20.3
Malathion	330.4	125	93	173	29.9
Chlorpyrifos	350.6	199	314	316	30.3
Triazofos	313.3	161	77	162	34.7
Terbuthylazin *	229.7	214	41	216	23.1
Carbofuran I	221.3	164	149	103	9.5
Carbofuran II	221.3	164	149	91	21.1
Trifluralin	335.3	306	264	41	19.1
Permethrin I	391.3	183	163	127	38.6
Permethrin II	391.3	183	163	127	38.8
Cypermethrin I	416.3	163	181	165	40.0
Cypermethrin II	416.3	163	181	165	40.2
Cypermethrin III	416.3	163	181	165	40.4
Deltamethrin	505.2	181	253	251	43.7
Naphthalin-D8 **	136.0	136	108	76	7.5

HCH: Hexachlorocyclohexane, * internal Standard, ** recovery standard.

2.4.2.2 Heavy metals

Heavy metals were analysed as described by McGrath and Cuncliffe (1985). Acids were purchased from Merck, Darmstadt, Germany and water was deionised (> 18 MΩ). All glassware was conditioned in a bath of 0.5 M nitric acid for at least six hours, rinsed with water and dried before use. All samples were analysed in duplicate.

3 g of oven-dried (40 °C), finely ground soil were weighed into round-bottomed reaction flasks and moistened with 2 mL of water. 21 mL of hydrochloric acid (32 %) and 7 mL of nitric acid (65 %) were added dropwise. The samples were allowed to stand overnight at room temperature and then boiled for 2 h using an aqua regia digestion apparatus with reflux condenser (Gerhardt, Königswinter, Germany). After cooling down to room temperature the samples were filtered through folded filters (Schleicher & Schuell, 512 ½) into 100 mL volumetric flasks, which were filled up with 0.5 M nitric acid. The heavy metals cadmium, copper, iron, lead, manganese, nickel and zinc were measured with Inductively Coupled Plasma – Mass Spectrometry (ICP-MS, Agilent 7500 Series). For the samples of the field experiment only a determination of copper due to the fungicide application of copper oxychloride was performed.

2.4.3 Functional parameters

2.4.3.1 Enzyme activities

All chemicals were purchased from Fluka, Taufkirchen, Germany. Every sample was analysed in duplicate plus one blank sample. Furthermore, two reagent blanks were determined. All solutions were prepared in distilled water. Because of light sensitivity of the enzyme reaction substrates and products for dehydrogenase, arylsulfatase and phosphatase activity determination every procedure was performed under diffused light.

2.4.3.1.1 Acid phosphatase

The analysis was performed according to Tabatabai and Bremner (1969). A universal buffer solution as described by Skujinš *et al.* (1962) was prepared as follows: 3.0 g of tris hydroxymethyl aminomethane, 2.9 g of maleic acid, 3.5 g of citric acid, 1.6 g of boric acid and

122 mL of 1 M sodium hydroxide (NaOH) solution were dissolved and filled up to 250 mL with distilled water. In order to prepare the buffer solution this stock solution was diluted 1:5 with distilled water and adjusted to pH 6.5 with 6 M hydrochloric acid.

1 g of field-moist soil was merged with 4 mL of buffer solution and 1 mL of substrate solution (25 mM sodium p-nitrophenyl phosphate in buffer solution) in glass centrifuge vials. Only 5 mL of buffer solution were added to the vials containing 1 g of field-moist soil for blank samples. The vials for the reagent blanks contained 1 g of quartz sand, 4 mL of buffer solution and 1 mL of substrate solution. Every vial was vortexed and incubated for 1 h in a shaking water bath at 37 °C. Thereafter 1 mL of 0.5 M calcium chloride ($CaCl_2$) solution and 4 mL of 0.5 M NaOH solution were added to stop the enzyme reaction and to form the product p-nitrophenol (p-NP). Then the vials were vortexed and centrifuged at 4000 rpm for 15 min. If necessary, the supernatants were diluted with a solution mixture (buffer solution, 0.5 M $CaCl_2$ solution, 0.5 M NaOH solution 5/1/4 (v/v/v)) and measured at a spectral photometer (Varian Cary 100) at a wavelength of 400 nm. The phosphatase activity was calculated by comparing absorbance values of the samples with those of standards ranging from 0 to 150 µg p-NP and are expressed in [g p-NP kg^{-1} h^{-1}].

2.4.3.1.2 Arylsulfatase

The analysis was performed according to Tabatabai and Bremner (1970). 1 g of field-moist soil was merged with 4 mL of buffer solution (0.5 M sodium acetate solution adjusted to pH 5.8 with glacial acetic acid) and 1 mL of substrate solution (25 mM potassium p-nitrophenyl sulfate in buffer solution) in glass centrifuge vials. Only 5 mL of buffer solution were added to the vials containing 1 g of field-moist soil for blank samples. The vials for the reagent blanks contained 1 g of quartz sand, 4 mL of buffer solution and 1 mL of substrate solution. Every vial was vortexed and incubated for 1 h in a shaking water bath at 37 °C. Then 1 mL of 0.5 M calcium chloride ($CaCl_2$) solution and 4 mL of 0.5 M sodium hydroxide (NaOH) solution were added to stop the enzyme reaction and to form the product p-nitrophenol (p-NP). Thereafter the vials were vortexed and centrifuged at 4000 rpm for 15 min. If necessary, the supernatants were diluted with a solution mixture (buffer solution, 0.5 M $CaCl_2$ solution, 0.5 M NaOH solution 5/1/4 (v/v/v)) and measured at a spectral photometer

(Varian Cary 100) at a wavelength of 400 nm. The arylsulfatase activity was calculated by comparing absorbance values of the samples with those of standards ranging from 0 to 150 µg p-NP and are expressed in [g p-NP kg^{-1} h^{-1}].

2.4.3.1.3 Cellulase

The analysis was performed according to Hope and Burns (1987). 1 g of field-moist soil was merged with 5 mL of buffer solution (0.1 M sodium acetate solution adjusted to pH 5.5 with glacial acetic acid) and 0.5 g of microcrystalline cellulose (Avicel, ~ 50 µm) in glass centrifuge vials. Only 5 mL of buffer solution were added to the vials containing 1 g of field-moist soil for blank samples. The vials for the reagent blanks contained 1 g of quartz sand, 5 mL of buffer solution and 0.5 g of Avicel. Every vial was vortexed and incubated for 16 h in a shaking water bath at 40 °C with exception of those of the blank samples, which were only incubated for 30 min. This was done because samples normally contain cellulose, which would act as substrate and lead to calculation mistakes. Then the vials were centrifuged at 4000 rpm for 15 min. Afterwards the supernatants were shaken end-over-end for 30 min with a potassium saturated cation exchange resin to remove disturbing metals for the subsequent colourimetric procedure for the determination of reducing sugars according to Schinner and von Mersi (1990), which was performed as follows:

1 mL of the supernatants was diluted to 10 mL with distilled water. For varying cellulase activity the dilution volume had to be adjusted according to the standards (see below). 1 mL of this dilution was merged with 1 mL of reagent A (0.09 % potassium cyanide in 1.6 % sodium carbonate solution) and 1 mL of reagent B (0.05 % potassium ferric hexacyanide solution) and heated for 15 min at 100 °C in screw capped test tubes. After cooling down to room temperature, 5 mL of reagent C (0.15 % ferric ammonium sulfate and 0.10 % sodium dodecyl sulfate in 0.42 % sulfuric acid solution) were added, the test tubes were vortexed and allowed to stand at room temperature for 1 h for complete colour formation. The cellulase activity was calculated by comparing absorbance values of the samples with those of standards ranging from 0 to 15 µg glucose, which were handled like the samples during the colourimetric procedure and are expressed in [mg glucose kg^{-1} h^{-1}].

2.4.3.1.4 Dehydrogenase

The analysis was performed as described by Malkomes (1993). 1 g of field-moist soil was merged with 2 mL of substrate buffer solution (0.5 % triphenyltetrazolium chloride (TTC) in buffer solution (0.1 M tris hydroxymethyl aminomethane solution, adjusted to pH 7.6 with 6 M hydrochloric acid solution)) in glass centrifuge vials. Only 2 mL of buffer solution were added to the vials containing 1 g of field-moist soil for blank samples. The vials for the reagent blanks contained 1 g of quartz sand and 2 mL of substrate solution. Every vial was vortexed and incubated for 24 h in a shaking water bath at 30 °C in the dark. Then 5 mL of acetone were added to stop the enzyme reaction and to extract the product triphenyl-formazane (TPF). Thereafter the vials were shaken end-over-end for 30 min and centrifuged at 4000 rpm for 15 min at 5 °C. If necessary the supernatants were diluted with a mixture of buffer solution and acetone 2/5 (v/v) and measured at a spectral photometer (Varian Cary 100) at a wavelength of 546 nm. The dehydrogenase activity was calculated by comparing absorbance values of the samples with those of standards ranging from 0 to 300 µg TPF and are expressed in [mg TPF kg^{-1} h^{-1}].

2.4.3.1.5 Urease

The analysis was performed according to Kandeler and Gerber (1988). 1 g of field-moist soil was merged with 4.5 mL of buffer solution (75 mM disodium tetraborate solution adjusted to pH 9.5 with 20 % sodium hydroxide (NaOH) solution) and 0.5 mL of substrate solution (80 mM urea in buffer solution) in glass centrifuge vials. Only 5 mL of buffer solution were added to the vials containing 1 g of field-moist soil for blank samples. The vials for the reagent blanks contained 1 g of quartz sand, 4.5 mL of buffer solution and 0.5 mL of substrate solution. Every vial was vortexed and incubated for 2 h in a shaking water bath at 37 °C. Then 6 mL of acidic potassium chloride (KCl) solution (0.01 M hydrochloric acid in 1 M KCl solution) were added to stop the enzyme reaction and to extract the product ammonium (NH_4^+). Thereafter the vials were shaken end-over-end for 30 min and centrifuged at 4000 rpm for 15 min. The supernatants were analysed for NH_4^+ by the following colourimetric procedure (Kandeler and Gerber, 1988):

1 mL of the supernatants was merged with 2 mL of distilled water, 2 mL of 0.1 % sodium dichloroisocyanurate solution and 5 mL salicylate solution (mixture of 0.3 M NaOH solution

and 0.12 % sodium nitroprusside in 17 % sodium salicylate solution 1/1 (v/v)). After vortexing the samples were allowed to stand at room temperature for 30 min for complete colour formation. If necessary, the samples were diluted with a solution mixture (buffer solution / acidic KCl solution (see above) (2/3; v/v), distilled water, salicylate solution (see above) and 0.1 % sodium dichloroisocyanurate solution 1/2/5/2 (v/v/v/v)) and measured at a spectral photometer (Varian Cary 100) at a wavelength of 400 nm. The urease activity was calculated by comparing absorbance values of the samples with those of standards ranging from 0 to 15 µg NH_4^+, which were handled like the samples during the colourimetric procedure and are expressed in [mg NH_4^+ kg^{-1} h^{-1}].

2.4.3.2 Respiratory parameters

2.4.3.2.1 Basal respiration

The analysis was performed according to Menyailo *et al.* (2003). Every sample was analysed in duplicate. 5 g of field-moist soil were placed in 60 mL incubation flasks and moistened with distilled water to 40 % of water holding capacity (WHC). The flasks were sealed with rubber stoppers and preincubated for 3 d at 28 °C. Thereafter the samples were moistened to 60 % of WHC, the flasks were closed with rubber stoppers, fixed with aluminium crimps and 6.5 mL ambient air were injected to set a pressure of about 100 mbar. 2.5 mL of headspace air were sampled before and after samples were incubated at 28 °C for 24 h. The headspace samples were transferred into autosampler vials with a gas-tight syringe and analysed for carbon dioxide (CO_2) by a gas chromatograph connected to an electron capture detector (GC/ECD, Shimadzu GC 14 A) via an autosampler (Dani HSS 1000).

The respiration rates expressed in [mg CO_2 kg^{-1} h^{-1}] were calculated by comparing peak areas of the samples with those of standards ranging from 0.01 to 1.0 % CO_2. The CO_2 concentrations before the incubation were subtracted as blanks from those afterwards to compensate the initial CO_2 concentrations in the flasks. For complete CO_2 ascertainment also CO_2 concentrations, which were dissolved physically using the Bunsen solubility coefficient (Equation **(1)**; Gmelin, 1974) and chemically using the pK value (Equation **(3)**; Gmelin, 1974) were calculated as follows.

$$[CO_2]_{phys} = \alpha \cdot V_{water} \cdot \frac{[CO_2]_{gas}}{MV_{act}} \cdot \frac{p_{act} + p_{over}}{p_{act}} \quad \textbf{(1)}$$

$[CO_2]_{phys}$ is the concentration of the physically dissolved CO_2, α is the Bunsen solubility coefficient for CO_2 (0.753), V_{water} is the volume of the soil water, MV_{act} is the actual mole volume calculated according to equation **(2)**, $[CO_2]_{gas}$ is the concentration of CO_2 in the gas phase, p_{act} is the actual atmospheric pressure and p_{over} is the set overpressure in the incubation flasks.

$$MV_{act} = \frac{T_{act} \cdot p_{norm} \cdot MV_{norm}}{T_{norm} \cdot p_{act}} \quad \textbf{(2)}$$

MV_{act} is the actual mole volume, T_{act} is the actual temperature, p_{norm} is the standard pressure (1013.25 mbar), MV_{norm} is the standard volume of 1 M gas (22414 mL), T_{norm} is the standard temperature (273.15 K) and p_{act} is the actual atmospheric pressure.

$$[CO_2]_{chem} = [CO_2]_{phys} \cdot 10^{pH-pK} \quad \textbf{(3)}$$

$[CO_2]_{chem}$ is the concentration of the chemically dissolved CO_2, $[CO_2]_{phys}$ is the concentration of the physically dissolved CO_2, pH is the pH value of the soil and pK is the pK value of H_2CO_3 (6.4).

2.4.3.2.2 Substrate-induced respiration

The analysis was performed according to Menyailo *et al.* (2003) as described for the basal respiration. Glucose was purchased from Fluka, Taufkirchen, Germany. The only difference between these analyses was as follows. After three days of preincubation 0.5 mL of 2.5 % aqueous glucose solution were added as carbon (C) source. The resulting concentration of glucose was 1 g C kg^{-1} soil. Thereafter the samples were also moistened to 60 % of water holding capacity and the analysis was proceeded as described above.

2.4.3.2.3 Microbial biomass

The analysis was performed using a modified method for substrate-induced respiration according to Menyailo *et al.* (2003) as described above. The differences between this method and the method used for the microbial biomass determination are both the preincubation and the incubation temperature, which were 22 °C as well as the incubation time, which was only 4 h. The microbial biomass (C_{mic}) expressed in [mg C (100 g)$^{-1}$] was computed through

the respiration rate (x) expressed in [mL CO_2 (100 g)$^{-1}$ h^{-1}] by means of equation **(4)** developed by Anderson and Domsch (1978): $C_{mic} = 40 \cdot x + 0.37$ **(4)**

2.4.3.2.4 Metabolic quotient

The metabolic quotient is the ratio between basal respiration and microbial biomass and is expressed in [mg CO_2 (g C_{mik})$^{-1}$ h^{-1}] (Anderson 2003).

2.4.3.3 Nitrogen parameters

2.4.3.3.1 Net nitrogen mineralisation

The analysis was performed according to Menyailo *et al*. (2003). All chemicals were purchased from Fluka, Taufkirchen, Germany. Every sample was analysed in duplicate.

15 g of field-moist soil were merged with 75 mL 1 M potassium chloride (KCl) solution in 100 mL plastic flasks. The flasks were shaken end-over-end for 1 h. The samples were filtered through folded filters (Schleicher and Schuell, 512 ½), if necessary, the filtrates were diluted with 1 M KCl solution and analysed with a continuous flow analyser (CFA, Skalar San+ System) for ammonium (NH_4^+) and nitrate (NO_3^-).

The determination of NH_4^+ is based on a modified Berthelot reaction. NH_4^+ is chlorinated to monochloramine, which reacts with salicylate to 5-aminosalicylate. After the oxidation and oxidative coupling a green coloured complex is formed. The concentrations of NH_4^+ were calculated by comparing the absorbance values measured at a wavelength of 660 nm of the samples with those of standards ranging from 0.05 to 0.90 µg NH_4^+ for low and from 0.5 to 10 µg NH_4^+ for high sample concentrations and are expressed in [mg NH_4^+-N kg^{-1}].

The determination of NO_3^- is based on a cadmium reduction method. The sample is passed through a column containing granulated cadmium to reduce the NO_3^- to NO_2^-, which is determined by diazotation with sulfanilamide and coupling with α-naphthylethylene-amine dihydrochloride to form a red coloured complex. The concentrations of NO_3^- were calculated by comparing absorbance values measured at a wavelength of 540 nm of the samples with those of standards ranging from 0.1 to 4.0 µg NO_3^- for low and from 3 to 30 µg NO_3^- for high sample concentrations and are expressed in [mg NO_3^--N kg^{-1}].

Further 15 g of field-moist soil were moistened to 60 % of water holding capacity (WHC) in 100 mL plastic flasks, which then were sealed with screw caps and incubated at 28 °C for 14 d. To avoid oxygen deficiency the flasks were opened every 3 d and vented for 5 min. At the end of the incubation the samples were analysed for NH_4^+ and NO_3^- as described above. The net nitrogen mineralisation rate was calculated as the difference between the sum of NH_4^+-N and NO_3^--N concentrations after and before the incubation and is expressed in [mg N_{min} kg^{-1} d^{-1}].

2.4.3.3.2 Net nitrification

The analysis was performed according to Menyailo *et al*. (2003) as described for the net nitrogen mineralisation but the net nitrification rate was calculated as the difference between NO_3^- concentrations after and before the incubation and is expressed in [mg NO_3^--N kg^{-1} d^{-1}].

2.4.3.3.3 Potential denitrification

The analysis was performed according to Menyailo *et al.* (2003). All chemicals were purchased from Fluka, Taufkirchen, Germany. Every sample was analysed in duplicate.

5 g of field-moist soil were placed in 60 mL incubation flasks and moistened with distilled water to 40 % of water holding capacity (WHC). The flasks were sealed with rubber stoppers and preincubated for 3 d at 28 °C. Thereafter 0.5 mL of 2.16 % potassium nitrate in 2.5 % aqueous glucose solution were added as nitrogen (N) and carbon (C) source and the samples were moistened to 60 % of WHC. The resulting concentration of nitrate was 0.3 g N kg^{-1} soil and that of glucose was 1 g C kg^{-1} soil. The flasks were closed with rubber stoppers and fixed with aluminium crimps. The headspace air was replaced by nitrogen to induce anaerobic conditions. 6.5 mL ethyne (approximates 10 % of headspace, generated from calcium carbide mixed with distilled water) were injected as an inhibitor of the nitrous oxide (N_2O) reductase and to set a pressure of about 100 mbar. 2.5 mL of headspace air were sampled before and after samples were incubated at 28 °C for 24 h. The headspace samples were transferred into autosampler vials and analysed for N_2O by a gas chromatograph connected to an electron capture detector (GC/ECD, Shimadzu GC 14 A) via an autosampler (Dani HSS 1000).

The potential denitrification rates expressed in [mg N_2O kg^{-1} h^{-1}] were calculated by comparing peak areas of the samples with those of standards ranging from 0.5 to 250 ppm N_2O. N_2O concentrations before the incubation were subtracted as blanks from those afterwards to eliminate the initial N_2O concentrations in the flasks. For complete N_2O ascertainment also the concentrations, which were dissolved physically, were calculated according to equations **(1)** and **(2)** (page 30). The value of the Bunsen solubility coefficient for N_2O was 0.544 (Gmelin 1974). N_2O is not chemically dissolved in water.

2.4.4 Structural parameters

2.4.4.1 Fatty acid nomenclature

Fatty acids are designated by the total number of carbon atoms. The degree of unsaturation is indicated by a number separated from the chain length by a colon. The following number divided by **ω** from that before explains the position of the double bonds from the methyl end of the molecule. The prefixes **a**, **i** and **cy** refer to anteiso-, iso- and cyclopropyl-branching fatty acids, respectively. A number followed by **Me** indicates the position of a methyl group from the carboxyl end of the molecule (Zelles, 1999).

2.4.4.2 Phospholipid fatty acid analysis

The following phospholipid fatty acids (PLFA) were determined in this study in the order of chromatographic retention: 14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω5c, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, 10Me17:0, 18:2ω6,9, 18:1ω9c, 18:ω7c, 18:0, 10Me18:0 and 20:4ω6 (Table 2). The abbreviation PLFA is synonymic to those fatty acids, which are separated from phospholipids during derivatisation while sample analysis (see below).

The analysis of PLFA is divided into three steps: **(1)** extraction, **(2)** fractionation and **(3)** derivatisation. The extraction was described by Frostegård *et al.* (1991), which is based on the method by Bligh and Dyer (1959) as modified by White *et al.* (1979). The fractionation was described by Frostegård *et al.* (1991), which is based on the method by King *et al.* (1977). The derivatisation is a modified method as described by Knapp (1979). Fatty acids are separated from phospholipids at first and then they are transformed to fatty acid methyl esters.

Table 2: Short forms, molecular weights, ion masses and retention times of (phospholipid) fatty acids (methyl esters) and standards used for quantification and identification in selected ion monitoring mode.

Phospholipid fatty acids (methyl esters)	Short forms	Molecular weights [g]	Target ions [m/z]	Qualifier ions 1 [m/z]	Qualifier ions 2 [m/z]	Retention times [min]
Nonadecanoic acid *	19:0	298.5	74	87	43	19.3
Myristic acid	14:0	228.4	74	87	43	10.5
13-Methylmyristic acid	i15:0	242.4	74	87	43	11.5
12-Methylmyristic acid	a15:0	242.4	74	87	43	11.7
Pentadecanoic acid	15:0	242.4	74	87	43	12.2
14-Methylpentadecanoic acid	i16:0	256.5	74	87	43	13.2
Palmitoleic acid	16:1ω7c	254.4	55	41	74	13.5
11-Hexadecenoic acid	16:1ω5c	254.4	55	41	74	13.7
Palmitic acid	16:0	256.4	74	87	43	13.9
10-Methylpalmitic acid	10Me16:0	270.5	74	87	43	14.6
15-Methylpalmitic acid	i17:0	270.5	74	87	43	14.9
14-Methylpalmitic acid	a17:0	270.5	74	87	43	15.1
9,10-Methylenepalmitic acid	cy17:0	268.5	55	41	74	15.4
Margaric acid	17:0	270.4	74	87	43	15.6
10-Methylmargaric acid	10Me17:0	284.5	74	87	43	16.3
Linoleic acid	18:2ω6,9	280.5	67	55	81	16.8
Oleic acid	18:1ω9c	282.5	55	41	69	16.9
Vaccenic acid	18:1ω7c	282.5	55	41	69	17.0
Stearic acid	18:0	284.5	74	87	43	17.4
10-Methylstearic acid	10Me18:0	298.5	74	87	43	18.1
Arachidonic acid	20:4ω6	304.5	79	67	80	20.2
Tridecanoic acid **	13:0	214.4	74	87	43	9.1

* internal Standard, ** recovery standard.

All organic solvents (residue- or HPLC-grade) were purchased from Promochem, Wesel, Germany and deionised water was used (> 18 MΩ). PLFA standards (purity > 98 %) were purchased by Larodan Fine Chemicals (Malmö, Sweden), Biotrend (Cologne, Germany), Fluka (Taufkirchen, Germany) and Sigma-Aldrich (Seelze, Germany). All glassware was rinsed with technical acetone and ethylacetate, washed at 95 °C with a detergent and heated at 300 °C overnight before use.

For extraction, 5 g of field-moist soil and 18.3 mL of a single phase mixture of chloroform-methanol-citrate buffer solution (0.15 M aqueous citrate acid solution) 1:2:0.8 (v/v/v) were merged in 100 mL glass centrifuge vials and closed with teflon-lined screw caps. The vials were shaken for 2 h at 225 rpm on a horizontal shaker, centrifuged at 4000 rpm for 15 min and thereafter the supernatants were transferred into separation funnels. An internal standard (15 µg 1.2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine in methanol) was added to the extracts. The remaining samples in the centrifuge vials were vortexed with further 5 mL of the single phase mixture, shaken for 1 h at 225 rpm on a horizontal shaker and centrifuged at 4000 rpm for 15 min. The supernatants were combined in the separation funnels and 6.2 mL of chloroform and 6.2 mL of citrate buffer solution were added in order to separate the organic from the water phase. The separation funnels were shaken for 30 min and left overnight for complete separation.

For fractionation, the lower chloroform phases were concentrated to 200 µL on a rotary evaporator (40 °C, 250 mbar). 0.5 g activated silica (silica 60, *reinst*-grade, 0.063–0.200 mm, Merck, Darmstadt, Germany) were slowly poured into a chromatographic column, which was filled with chloroform. The chloroform was drained until the level reached the top of the silica and then the samples were transferred onto the silica. The columns were eluted sequentially with 5 mL of chloroform (abolished), 20 mL of acetone (abolished) and 20 mL of methanol. The methanol phases were concentrated to 200 µL on a rotary evaporator (40 °C, 100 mbar), transferred into reaction vials with methanol and dried under nitrogen stream.

For derivatisation, 0.5 mL of 0.5 M methanolic sodium hydroxide solution were added to the samples, which then were boiled for 10 min at 100 °C. After cooling down at room temperature 0.75 mL of 13 % methanolic borontrifluoride solution were added to the samples, which thereafter were heated for 15 min at 80 °C. After cooling down at room temperature the samples were liquid-liquid extracted. For this purpose, 1 mL of hexane and - to enforce the transfer of the PLFA into the organic solvent phase - 0.5 mL of saturated sodium chloride solution were added. After shaking the vials on a horizontal shaker at 225 rpm for 10 min the upper hexane phases were transferred into other reaction vials. This separation procedure was repeated twice and after combining the hexane phases in reaction vials they were dried under nitrogen stream. Finally, a recovery standard (10 µg tridecanoic acid methyl ester in toluene) was added to determine the recovery of the internal standard.

The samples were transferred into autosampler vials with toluene and analysed by a gas chromatograph (GC) connected to a mass selective detector (MSD) (GC: Hewlett Packard (HP) Series II 6890; MSD: HP 6971 A) and an autosampler (HP 7673). The resulting fatty acid methyl esters were separated on a 30 m capillary column (HP-5 MS consisting of 5 % phenyl-methyl siloxan) with an inner diameter of 0.25 mm and a film thickness of 0.25 µm. Helium (purity: 99.996 %) was used as carrier gas with a column pressure of 84 kPa and 1 µL of sample solution was injected splitless (splitless time: 1.25 min). The injector temperature was 150 °C and the detector temperature 310 °C. The temperature program was run as follows: Initial temperature 80 °C held for 1 min, increased at 7 °C min^{-1} to 180 °C, increased at 1.3 °C min^{-1} to 195 °C held for 5.4 min, increased at 1.3 °C min^{-1} to 231 °C and finally increased at 50 °C min^{-1} to 300 °C held for 5 min. The ionisation was conducted by electron impact (70 eV) mode and the measurement was done in selected ion monitoring mode. The identification of the fatty acid methyl esters was based on comparison with retention times and mass spectra, which were obtained from standards (Table 2). The internal standard (consisting of two fatty acids 19:0 and a phospholipid) was used to compensate sample-processing losses, while the recovery standard was used to countervail apparatus-induced measurement variations and allowed to quantify the recoveries of the internal standards. The PLFA concentrations were calculated as outlined for pesticides by Laabs *et al.* (1999). The mentioned target ions are specific fragment ions per analyte with the highest abundance, while qualifier ions are two further characteristic ions after ionisation in the mass selective detector (Table 2).

2.5 Statistical analysis

2.5.1 Land use systems

The heavy metal results were analysed by one-way analysis of variance (ANOVA) using the various land use systems as independent variables. For this purpose, the data had to be transformed via various continuous functions in order to obtain normally distributed values (Backhaus and Erichson, 2003). The means were separated using least significant difference (LSD) test with a significance level of $P < 0.05$. An ANOVA for the pesticide values was not performed because not all pesticides were applied on every conventionally managed field.

All results of the soil microbial community functional parameters were analysed by one-way ANOVA as described for heavy metals. With the aid of a Kaiser normalised and Varimax rotated principal component analysis (PCA) with correlation matrix using all soil microbial function parameters as variables the data were reduced and correlating parameters were combined into independent components. The factor scores were extracted by the regression method and Eigenvalues greater than 1. By means of a subsequent discriminant analysis using the land use systems as grouping variables and the factor scores of the PCA as independent variables the soils were allocated into groups in order to ascertain whether it is possible to distinguish soils of different land use systems. Moreover, a hierarchical cluster analysis was conducted using the factor scores of the PCA as variables and the land use systems as label cases in order estimate similarities between the land use systems.

With the aid of a Kaiser normalised and Varimax rotated PCA with correlation matrix using all soil microbial community structural parameters (phospholipid fatty acids (PLFA) expressed in relative abundances of the total PLFA content) as variables the correlating PLFA were combined into independent components in order to discriminate between different microbial taxonomic groups. Values of saturated, non-branched PLFA (14:0, 15:0, 16:0, 17:0 and 18:0) were excluded from the PCA because these PLFA are constituents of all microbial cell membranes and thus the discrimination between various groups is not possible (Zelles, 1999). The factor scores were extracted by the regression method and Eigenvalues greater than 1. Due to the same objectives as described for the functional parameters a discriminant analysis and a hierarchical cluster analysis were conducted as explained above. The absolute and relative abundances of the PLFA sum contents for all microbial groups resulting from the PCA as well as the total PLFA content were analysed by a one-way ANOVA as described for heavy metals.

Finally, a correlation matrix was calculated using the soil microbial community structure (PLFA sum contents of the individual microbial taxonomic groups) and function parameters. The significance levels ($P < 0.05$ and $P < 0.01$) of the correlation matrix were based on the Spearman correlation coefficient. All statistical analyses were performed with SPSS 10.0 for Windows.

2.5.2 Field experiment

The pollutant (copper and pesticides) results were analysed by one-way ANOVA using the various treatments as independent variables. This analysis was conducted as described for heavy metal investigation in the land use system samples (see 2.5.1, page 34).

All results of the soil microbial community functional parameters were analysed by one-way ANOVA as described for pollutants. A PCA was conducted with soil microbial function parameters as described for the land use system sites (see 2.5.1, page 34). With the aid of a subsequent discriminant analysis using the different treatments at respective sampling dates as grouping variables and the factor scores of the PCA as independent variables the soils were allocated into groups in order to ascertain whether it is possible to distinguish soils of the different treatments at all sampling dates.

A PCA was conducted for the phospholipid fatty acids (PLFA) expressed in relative abundances of the total PLFA content as described for the land use system sites (see 2.5.1, page 34). Due to the same objectives as defined for the functional parameters a discriminant analysis was performed as described above. The absolute and relative abundances of the PLFA sum contents for all microbial groups resulting from the PCA as well as the total PLFA content were analysed by a one-way ANOVA as described for pollutants.

Finally, a correlation matrix was calculated using the soil microbial community structure (PLFA sum contents of the individual microbial taxonomic groups) and function parameters as described for the land use system sites (see 2.5.1, page 34). All statistical analyses were performed with SPSS 10.0 for Windows.

3. Results and discussion

3.1 Land use systems

3.1.1 Basic soil parameters

Texture, which ranged between loamy or clayey silt and silty loam or clay, did not vary a lot between soils of the diverse land use systems (Table 3). Agricultural field soils had similar values for the remaining basal parameters. However, reference soils showed a significantly ($P < 0.05$) lower pH value and a significantly ($P < 0.05$) higher C/N ratio due to no carbonate and nitrogen fertilisation, respectively, compared to agricultural field soils. Hence, the higher C/N ratio in fallow soils was also owing to abandoned manuring. Pasture and reference soils exhibited significantly ($P < 0.05$) higher TOC and TON contents caused by higher dung and litter input, respectively, while nutrients were discharged by harvest on agricultural fields.

Table 3: Basic properties of the investigated soils under diverse land use systems (± standard errors).

Land use systems	Sand [%]	Silt [%]	Clay [%]	pH [---]	TOC [g kg^{-1}]	TON [g kg^{-1}]	C/N [---]
Conv. agr. field	8.8 ± 0.8	62.5 ± 2.8	29.8 ± 2.9	7.1 ± 0.2	23.0 ± 1.7	2.2 ± 0.1	10.3 ± 0.2
Conv. greenh.	8.6 ± 0.8	61.1 ± 1.3	31.7 ± 2.4	7.4 ± 0.3	24.1 ± 1.1	2.0 ± 0.1	10.0 ± 0.1
Org. agr. field	10.9 ± 2.3	64.3 ± 1.0	25.3 ± 1.6	7.1 ± 0.3	23.2 ± 2.0	2.2 ± 0.1	10.3 ± 0.5
Org. greenh.	9.4 ± 1.9	68.4 ± 2.4	23.1 ± 3.1	7.5 ± 0.2	24.6 ± 3.5	2.3 ± 0.3	9.9 ± 0.2
Fallow	9.0 ± 1.4	62.6 ± 1.1	28.9 ± 2.8	7.0 ± 0.2	22.0 ± 1.3	2.0 ± 0.1	10.9 ± 0.2
Pasture	6.9 ± 0.5	66.3 ± 1.5	26.8 ± 1.9	6.7 ± 0.2	29.9 ± 3.6	2.8 ± 0.4	10.4 ± 0.3
Reference	4.3 ± 0.0	62.9 ± 0.0	33.7 ± 0.0	5.7 ± 0.1	36.6 ± 1.0	3.1 ± 0.1	11.7 ± 0.3

pH: pH value in distilled water, TOC: total organic carbon, TON: total organic nitrogen, C/N: carbon/nitrogen ratio; conv.: conventional, org.: organic, greenh.: greenhouse, agr.: agricultural.

3.1.2 Pollutants

3.1.2.1 Pesticides

Figure 3 shows the average pesticide concentrations in soils of the conventional land use systems. No mean contents for the same systems such as agricultural field, greenhouse or

fallow were computed because different pesticides were applied on individual sites. Hence, a calculation of means of the same land use systems would lead to an underestimation of the real pesticide contamination situation within the investigation area. With the exception of cypermethrin, deltamethrin, malathion and triazofos, which were below the detection limit of 0.1 μg kg^{-1} in every soil sample, all other investigated pesticides could be quantified but exclusively in soils of conventionally managed fields and greenhouses (Figure 3). The most abundant pesticide endosulfan and its metabolite endosulfan sulfate were also detected in a fallow soil, which was previously used as a conventionally cultivated field two years ago. The concentrations of carbofuran, chlorpyrifos, chlorothalonil, dimethoate, endosulfan, endosulfan sulfate, permethrin and trifluralin varied between 0 and 34.2 μg kg^{-1} (Figure 3). In soils of the remaining sites the pesticide contents were below the detection limit, which is an evidence that only on conventionally cultivated fields chemical pest control was performed. As expected endosulfan sulfate, α- and β-endosulfan correlated mutually highly significantly ($P < 0.01$, $R = 0.600, 0.715, 0.920$) because technical endosulfan, which is applied to the fields, is a mixture of the two isomers at a ratio of about 7:3 (Siddique *et al*., 2003) and in soils these isomers are degraded to the highly toxic endosulfan sulfate (Antonious and Byers, 1997).

Miglioranza *et al*. (1999) reported concentrations of seven organochlorine pesticides (OCP) and metabolites between 0 – 60 μg kg^{-1} in natural and 0 – 100 μg kg^{-1} in horticultural soils of Typic Argiudolls of the *Los Padres* watershed in Buenos Aires Province, Argentina. Miglioranza *et al*. (2002) found OCP contents between 6.7 – 32.6 μg kg^{-1} in 0 – 30 cm soil depth of an agricultural farm in the southeastern region of Buenos Aires Province. Gonzalez *et al*. (2003) analysed total bulk soil concentrations of endosulfan and endosulfan sulfate of 16.5 μg kg^{-1} in untreated soils from an agricultural environment in the southeastern region of Buenos Aires province. Miglioranza *et al*. (2004b) determined OCP concentrations in fluvic sediments in the range between 6 and 25 μg kg^{-1} with endosulfan as the most abundant compound. Jergentz *et al.* (2005) found 0.1 – 150, 13 – 46 and 7.8 μg kg^{-1} of chlorpyrifos, cypermethrin and endosulfan in sediments of small streams in the main soybean area of Argentina, respectively. The pesticide contents described in these studies correspond to those of the present investigation but mostly only OCPs were analysed. With respect to the study area around Buenos Aires no further data were found about the remaining pesticides determined in this investigation so that no more comparison to literature data was possible.

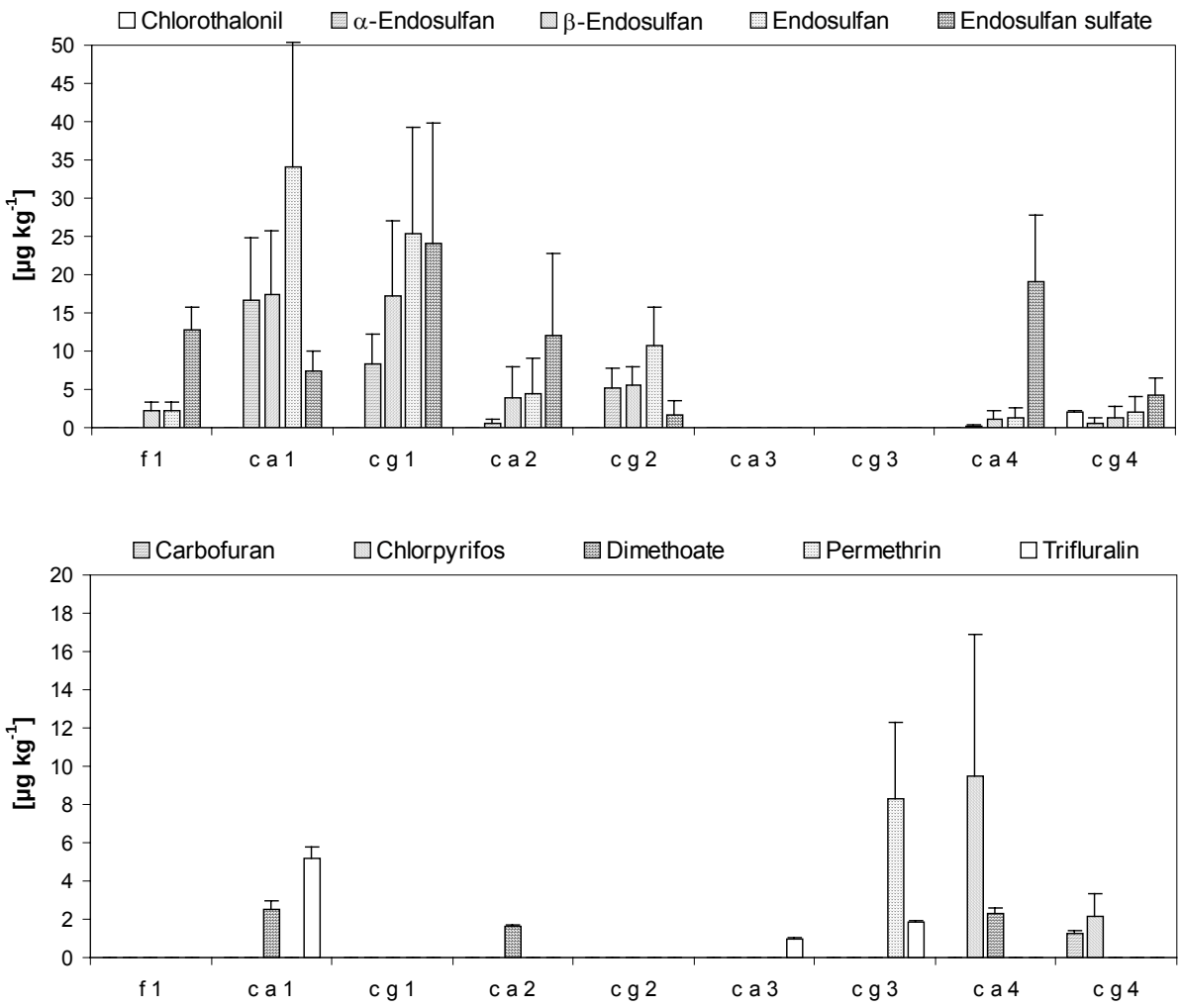

Figure 3: Average pesticide concentrations in soils of the conventional land use systems under study with standard error bars; endosulfan is the total of α- and β-endosulfan; c: conventional, a: agricultural field, g: greenhouse, f: fallow, 1 – 4: site replications.

Table 4 shows pesticide thresholds of the Dutch List (1994) for soils and sediments for a standard Dutch soil (10 % organic matter and 25 % clay). These thresholds are intervention values that signal serious soil contamination when exceeded and are based upon human and ecotoxicological criteria. Since these values of the Dutch List are related to other pesticides (besides carbofuran) it would not be correct to equate them with those determined in the present study. However, they provide approximate benchmarks to estimate environmental risk potentials of the analysed pesticide contamination. The threshold for carbofuran of the Dutch List (2 mg kg^{-1}, Table 4) is three orders of magnitude higher than the level of carbofuran of the present study (1.3 µg kg^{-1}). Similarly, total thresholds of the Dutch List for chlorinated pesticides like hexachlorocyclohexanes (2 mg kg^{-1}, Table 4) and drins (4 mg kg^{-1},

Table 4) are at least two orders of magnitude higher compared to the concentrations of endosulfan (maximum: 34.2 μg kg^{-1}, Figure 3). Based on the comparison of pesticide contents determined in this study and the thresholds for soils and sediments from the Dutch List no risk potential is expected from pesticide contamination for the environment of the inner zone of the green belt around Buenos Aires at the moment. However, it is strongly suggested to further monitor pesticide contents to avoid a pesticide accumulation when the intensive pesticide application is maintained in the future. Usually pesticides are applied to soils between 0.5 and 2.0 kg ha^{-1} of the active ingredient in Argentinean horticulture, which correspond to theoretical concentrations between 0.4 and 1.6 mg of active ingredient kg^{-1}. Assuming that only 1 % of the active agent is absorbed by target organisms (Andrade, *et al.*, 2005) when compared to the concentrations determined in this study (Figure 3) there is a great difference between these levels. Pesticides are lost from soils by physical processes (volatilisation, leaching, erosion), chemical and microbial degradation or they are fixed to soil particles as bound residues (Gevao *et al.*, 2000), which could be the reason for the low concentrations analysed in this study.

Table 4: Limit values of pesticide concentrations [mg kg^{-1}] of the Dutch List (1994) for soils and sediments for a standard Dutch soil (10 % organic matter and 25 % clay) and the analysed data for carbofuran [mg kg^{-1}] of the present investigation.

Pesticides	DDT, -D, -E	*Drins*	HCH	Carbaryl	Carbofuran	Maneb	Atrazin
Dutch List	4	4	2	5	2	35	6
Analysed data	n. d.	n. d.	n. d.	n. d.	0.0013	n. d.	n. d.

n. d.: not detected, DDT, -D, -E: total of DDT, DDD, DDE, *Drins*: total of Aldrin + Dieldrin + Endrin
HCH: total of α, β-, γ- and δ-hexachlorocyclohexane.

3.1.2.2 Heavy metals

The average contents of the determined heavy metals in soils of every land use system are shown in Figure 4. With the exception of Cd (detection limit: 0.7 mg kg^{-1}) all soil samples contained detectable heavy metal contents. They showed a decreasing abundance in the order Fe > Mn > Zn > Cu > Pb > Ni > Cd. The latter was detected only in one of the fallow sites with an average content of 0.9 mg kg^{-1}. Mean contents in the upper 10 cm of the remaining heavy metals are 18 g kg^{-1} of Fe and 0.7 g kg^{-1} of Mn, while Zn, Cu, Pb and Ni

concentrations are 37, 18, 11 and 7 mg kg^{-1}, respectively. No significant differences ($P < 0.05$) could be identified between the diverse agricultural land use systems. Cu, Fe, Ni and Pb contents correlated highly significantly ($P < 0.01$). Zn correlated significantly ($P < 0.05$) with Fe and Ni and highly significantly ($P < 0.01$) with Cu. Mn exhibited only significant correlations ($P < 0.05$) to Pb and Cu and there was no correlation ($P > 0.05$) between Pb and Zn. No relationships were found between heavy metals and soil texture and pH values.

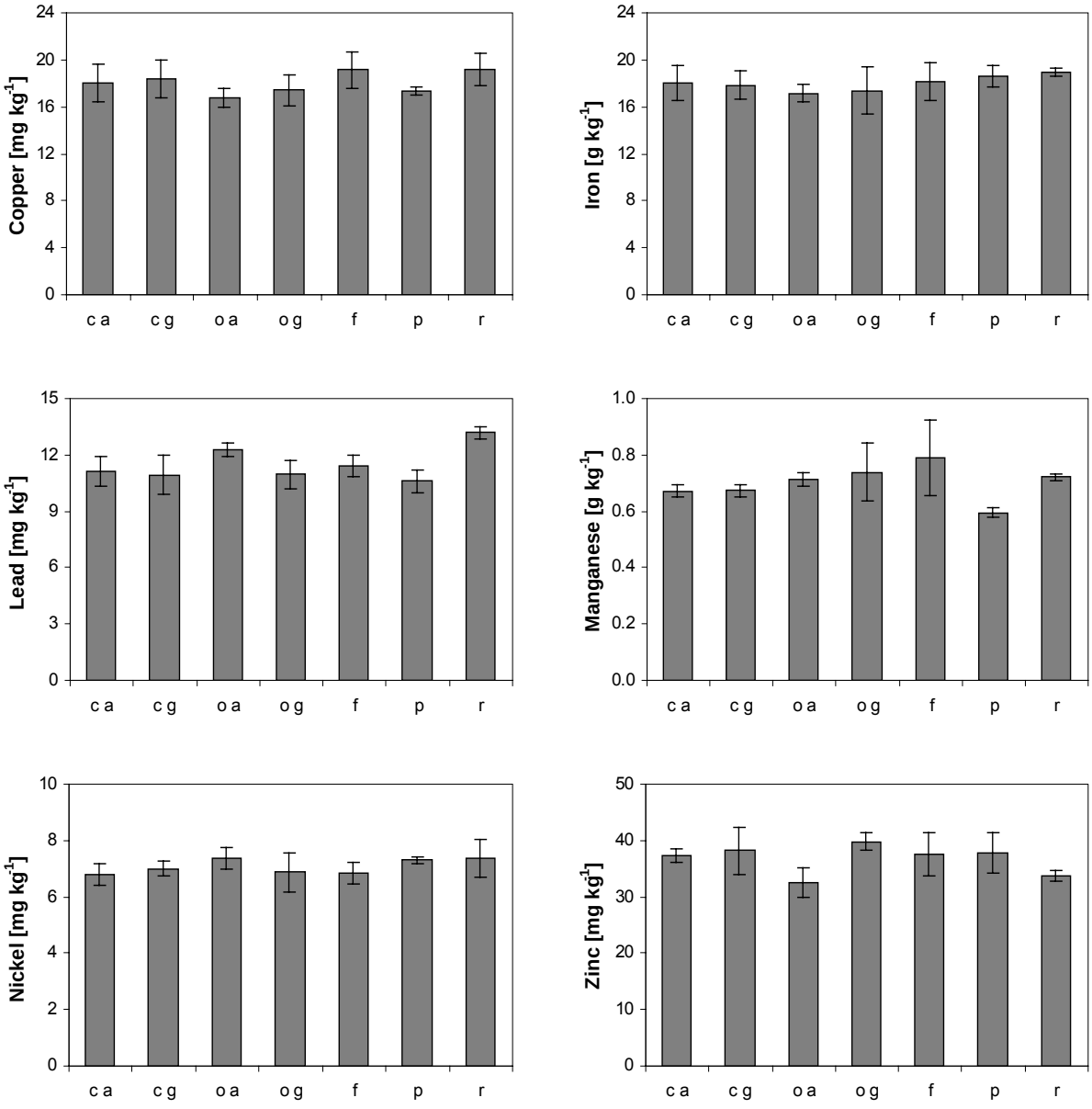

Figure 4: Average heavy metal contents in soils of the land use systems under study with standard error bars; c: conventional, o: organic, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference.

The system soil-air-water is connected to various exchange processes in the environment. Naturally occurring heavy metals are entered into soils over dry and wet deposition from the atmosphere and are discharged by wind and water erosion through soil constituents or leached into ground water. In surface waters or soils they could be dissolved from the earth crust, sprayed into the atmosphere through aerosols or sedimented bound to particles (Alloway, 1995). In general, to estimate heavy metal inputs into soils and to assess the thereby anthropogenic contribution every of these input pathways have to be taken into account. The reference soil exhibited the highest concentrations of Cu, Fe, Pb and Ni and no significantly ($P > 0.05$) lower contents of Mn and Zn in comparison to the other land use systems. As this anthropogenically unaffected soil constitutes natural background levels for heavy metal contamination in soils of the inner zone of the green belt around Buenos Aires it can be assumed that also the agriculturally managed soils only represent background levels. Therefore, significant anthropogenic sources for heavy metals could be excluded. The slightly but not significantly ($P > 0.05$) lower Cu, Fe, Pb and Ni levels of the cultivated soils compared to the reference soils are likely due to heavy metal exportation into the cultural crops, which should be the focus of further studies.

Ronco *et al*. (2001) investigated heavy metal contents from stream bottom sediments of the Río de la Plata estuary. It was shown that the coastal plain sector behaves as a regional sink between the upstream area and the estuary. The authors reported that varying and partially high contents of Cd, Cu, Pb and Zn (Table 5) - especially in the polluted *Del Gato* stream - among different streams within the same geomorphological and geological structures indicate anthropogenic sources of heavy metals. Camilión *et al*. (2003) indicated that bottom sediment heavy metal concentrations of the south-western coast of the Río de la Plata estuary are locally related to neighbouring metal topsoil contents within the basin whereby the values in soils are higher than in sediments. The Argiudolls in that study region are frequently affected by rainwater erosion. Thus, the fine materials with sorbed heavy metals are washed into the rivers, which carry them downstream where the particles are sedimented (Camilión *et al*., 2003). Therefore, higher contents of Cu, Pb and Zn (Table 5) compared to those in the present study were not found in the upstream areas, where some of the sampling sites were located, but mainly in the lower plains. Bilos *et al*. (2001) published low concentrations of airborne particulate trace metals of sites located in residential,

industrial and commercial sectors of La Plata City area. Concentrations of Cd, Cu, Fe, Mn, Ni, Pb and Zn, also analysed in the present study, are comparable to values reported for cities, which are not heavily polluted such as Birmingham (United Kingdom) or Mallipo (South Korea) and are below the general concentrations described for urban particulates (Bilos *et al*., 2001). Via enrichment factors the authors assumed that Pb, Zn, Cu and Cd are not only derived from natural, but supplementary from anthropogenic sources such as motor exhausts. According to the results of the present and the cited studies (Table 5) - besides the hot spot in the *Del Gato* stream (Ronco *et al*., 2001) - no alarming contents of heavy metals could be found in stream sediments, soils or the atmosphere of the study area.

Table 5: Mean heavy metal contents from stream sediments and topsoils with standard deviations, number of sampling sites as well as medians, minimum and maximum contents of the study of Ronco *et al.* (2001), Camilion *et al.* (2003) and the present investigation.

	Means	Std. dev.	Sites	Medians	Minima	Maxima
Ronco *et al.* (2001)			**S e d i m e n t s**			
Copper [mg kg^{-1}]	28.7	27.6	62	68.5	4.0	133.0
Lead [mg kg^{-1}]	34.4	50.0	62	106.5	1.0	212.0
Zinc [mg kg^{-1}]	86.8	152.0	62	351.8	0.5	703.0
Cadmium [mg kg^{-1}]	0.65	0.35	62	1.40	0.50	2.25
Nickel [mg kg^{-1}]	11.9	8.5	60	17.2	0.5	35.2
Iron [g kg^{-1}]	27.7	24.9	55	61.1	0.1	122.0
Manganese [g kg^{-1}]	0.50	0.37	55	0.80	0.01	1.54
Camilion *et al.* (2003)			**S o i l s**			
Copper [mg kg^{-1}]	32.1	21.6	64	80.0	4.9	155.0
Lead [mg kg^{-1}]	68.4	69.3	64	156.5	2.0	311.0
Zinc [mg kg^{-1}]	118.2	74.2	64	181.3	17.6	345.0
Present investigation			**S o i l s**			
Copper [mg kg^{-1}]	17.9	2.4	23	18.2	14.0	22.3
Lead [mg kg^{-1}]	11.3	1.4	23	11.2	9.0	13.3
Zinc [mg kg^{-1}]	36.9	5.6	23	36.9	27.5	46.2
Cadmium [mg kg^{-1}]	0.04	0.19	23	0.45	0.00	0.90
Nickel [mg kg^{-1}]	7.0	0.7	23	7.2	6.0	8.3
Iron [g kg^{-1}]	17.9	2.3	23	18.8	15.0	22.5
Manganese [g kg^{-1}]	0.70	0.13	23	0.88	0.57	1.18

Std. dev.: standard deviation.

De López Camelo *et al*. (1997) mentioned that the application of mineral fertilisers used in Argentinean agriculture such as rock phosphates could be responsible for higher amounts of Cd and Zn, superphosphate for Pb and diammonium phosphate for chrome and Cu in the soil. The authors reported that contents of heavy metals varied considerably and that the levels of Cd and Pb in some analysed fertilisers were significant relative to those naturally present in soils. Lavado *et al*. (1999) found higher contents of cobalt, Cu, Ni and Pb extracted with diethylenetriaminepentaacetic acid in cropped soils compared to pasture soils of the Pampa region of Pergamino (33° 56′ S, 60° 34′ W) due to the application of trace element enriched mineral phosphate fertilisers. In contrast, in their study, arsenic, Fe, Mn and Zn showed lower contents in cropped than in the pasture soils because of uptake by plants as micronutrients and exportation by harvest. By reason of no significant differences in heavy metal contents in soils of the various land use systems no particular sources for heavy metals caused by different agricultural practice could be assumed.

The concentrations of elements with high environmental risk potential such as Cd, Cu, Ni and Pb were lower than the admissible contents for soils of the German soil conservation regulation (BBodSchV, 1999) and the Dutch List (1994) (Table 6). The heavy metal contents were lower than outlined by Morrás *et al.* (1998) (Table 7) who studied the geochemical composition of Argiudolls and loess sediments in Castelar (northeastern of the province of Buenos Aires) in the Pampa Ondulada and were partly only half of the agricultural soils analysed by Lavado *et al.* (1998) (Table 7) who examined heavy metals in Mollisols in Buenos Aires City and province. Compared to the investigation of Lavado *et al.* (2004), who analysed background levels of potentially toxic elements in Pampean Mollisols, the present study showed lower contents of Cd, Pb and Zn, but similar values of Ni and Cu and slightly higher ones for Mn (Table 7) than those found in Typic Argiudolls by these authors. Hence, it is reasoned that the determined heavy metal contents are also background levels for the soils of the inner zone of Buenos Aires province.

Table 6: Thresholds of heavy metal concentrations [mg kg^{-1}] in grassland soils of the German soil conservation regulation (BBodSchV, 1999) from aqua regia dissolution for the pollutant transfer between soil and crops and of the Dutch List (2004) for soils and sediments for a standard Dutch soil (10 % organic matter and 25 % clay). In addition, the highest analysed data for heavy metals [mg kg^{-1}] of the present investigation.

Heavy metals	Copper	Lead	Zinc	Cadmium	Nickel	Arsenic	Mercury
BBodSchV	1300	1200	n. d.	20	1900	50	2
Dutch List	190	530	720	12	210	55	10
Analysed data	22	13	46	1	8	n. d.	n. d.

BbodSchV: *Bundesbodenschutzverordnung* (German soil conservation regulation); n. d.: no data.

Table 7: Mean heavy metal contents from topsoils with standard deviations of the study of Morrás *et al.* (1998), Lavado *et al.* (1998) and Lavado *et al.* (2004) and of the present investigation.

	Copper [mg kg^{-1}]	Lead [mg kg^{-1}]	Zinc [mg kg^{-1}]	Cadmium [mg kg^{-1}]	Nickel [mg kg^{-1}]	Iron [g kg^{-1}]	Manganese [g kg^{-1}]
Morrás *et al.* (1998)	24.0 ± 1.7	25.7 ± 13.1	53.7 ± 8.4	n. d.	5.3 ± 2.5	n. d.	0.86 ± 0.06
Lavado *et al.* (1998)	30.0 ± 1.5	38.0 ± 3.0	85.0 ± 2.0	12.00 ± 0.75	14.0 ± 2.0	n. d.	n. d.
Lavado *et al.* (2004)	18.0 ± 8.3	17.7 ± 6.0	48.0 ± 24.1	0.75 ± 0.46	7.3 ± 4.3	n. d.	0.60 ± 0.17
Present investigation	17.9 ± 2.4	11.3 ± 1.4	36.9 ± 5.6	0.04 ± 0.19	7.0 ± 0.7	17.9 ± 2.3	0.70 ± 0.13

n. d.: no data.

3.1.3 Functional parameters

3.1.3.1 Enzyme activities

The acid phosphatase activity ranged between 0.41 and 1.22 g p-nitrophenol (p-NP) kg^{-1} h^{-1} (Figure 5A) and decreased in the order pastures > references > organic agricultural fields > conventional agricultural fields > organic greenhouses > fallows > conventional greenhouses. The acid phosphatase activity of the pasture soils was significantly ($P < 0.05$) higher than of the soils of all other land use systems. The organically managed soils exhibited a tendentially higher acid phosphatase activity than those of the conventionally managed ones, while the greenhouse soils had a lower acid phosphatase activity than those of the agricultural fields.

The acid phosphatase activity of the fallow soils did not differ from those of all cultivated soils, while the activity of the reference soils was slightly higher than those of the agricultural field ones. Deng and Tabatabai (1997) reported an acid phosphatase activity between 0.20 and 0.32 g p-NP kg^{-1} h^{-1} in soils of different tillage and residue managements, while de la Paz Jimenez *et al*. (2002) found an acid phosphatase activity of 0.36 – 1.26 p-NP kg^{-1} h^{-1} in Vertic Argiudolls of forestal, pastural and agricultural managements located at La Paz in Entre Rios Province, Argentina, being in the same order of magnitude as the reported values.

The arylsulfatase activity ranged between 0.11 and 0.41 g p-nitrophenol (p-NP) kg^{-1} h^{-1} (Figure 5B) and decreased in the order pastures > references > organic agricultural fields > organic greenhouses > fallows > conventional agricultural fields > conventional greenhouses. The pasture soils showed a significantly ($P < 0.05$) higher arylsulfatase activity than those of all other soils except for the reference ones, which exhibited a significantly ($P < 0.05$) higher arylsulfatase activity than the fallows and conventional agricultural fields and greenhouses. The arylsulfatase activity of the organically managed soils was tendentially higher than that of the conventionally managed ones, while the greenhouse soils had a tendentially lower arylsulfatase activity than those of the agricultural fields. The arylsulfatase activity of the fallow soils did not differ from those of all cultivated soils. Deng and Tabatabai (1997) found an arylsulfatase activity of 0.10 – 0.20 g p-NP kg^{-1} h^{-1} in soils of different tillage and residue managements, while de la Paz Jimenez *et al*. (2002) detected an arylsulfatase activity between 0.02 and 0.19 g p-NP kg^{-1} h^{-1} in Vertic Argiudolls of forestal, pastural and agricultural managements located at La Paz in Entre Rios Province, Argentina, which are in the same order of magnitude as the reported values.

The cellulase activity ranged between 8.0 and 15.4 mg glucose kg^{-1} h^{-1} (Figure 5C) and decreased in the order pastures > organic greenhouses > references > organic agricultural fields > conventional greenhouses > fallows > conventional agricultural fields. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. The organically managed soils exhibited a tendentially higher cellulase activity than the conventionally managed ones and the greenhouse soils showed higher values compared to the agricultural field ones. The cellulase activity of the fallow soils did not differ from those of all cultivated soils, while the pasture and reference soils exhibited the highest cellulase activity. Kandeler *et al*. (1996) reported a cellulase activity of 3.0, 3.2 and 4.7 mg glucose

kg^{-1} h^{-1} in a Dystric Lithosol, an Eutric Cambisol and a Calcaric Phaeozem, respectively, being 50 to 80 % lower than the values in the present study. Omar and Abdel-Sater (2001) found a cellulase activity between 5.5 and 11.4 mg glucose kg^{-1} h^{-1} in a botanical garden soil in Assiut, Egypt, being in the same order of magnitude as the reported values.

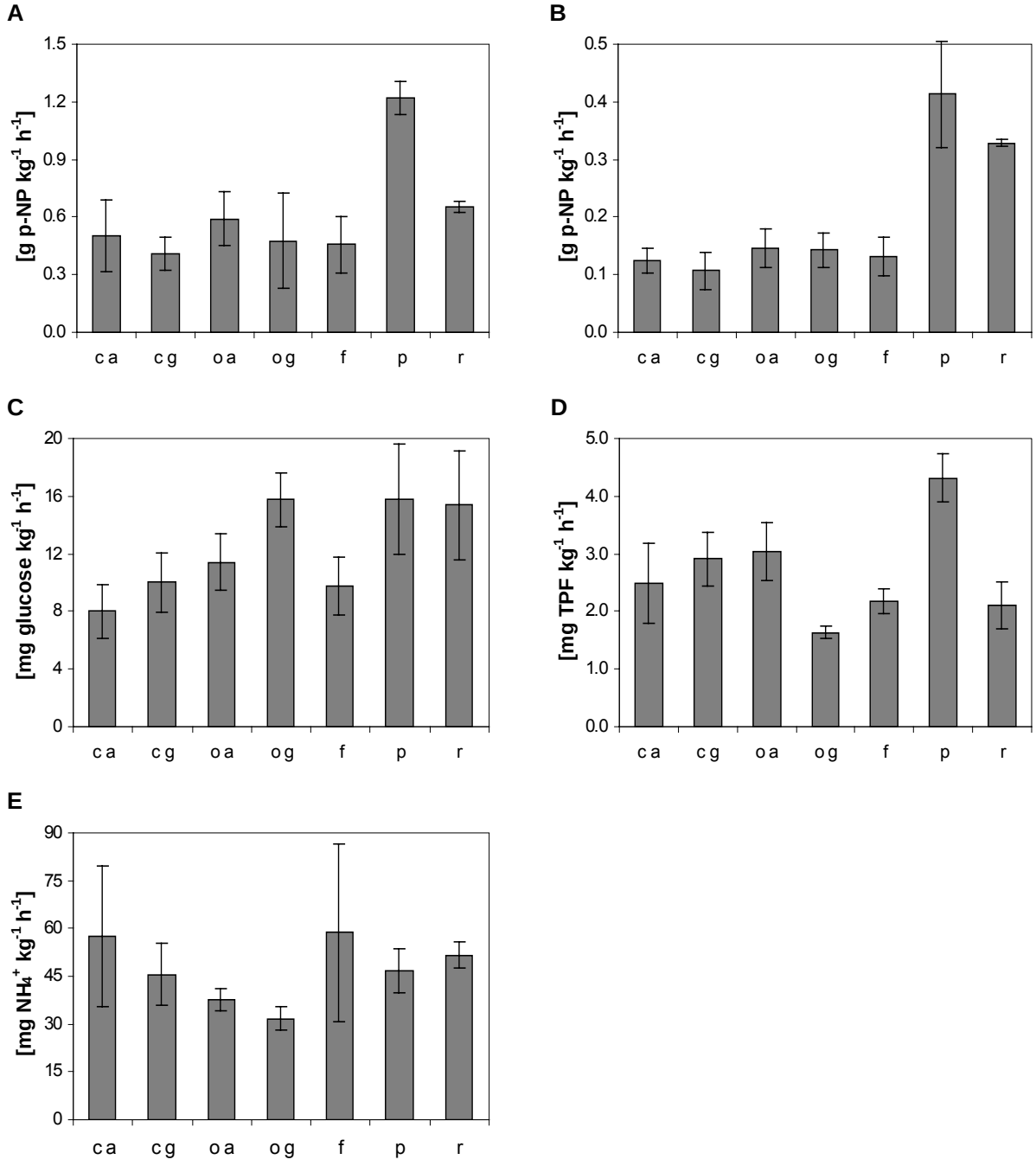

Figure 5: Average enzyme activities in soils of the land use systems under study with standard error bars; c: conventional, o: organic, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference; **A**: acid phosphatase, **B**: arylsulfatase, **C**: cellulase, **D**: dehydrogenase, **E**: urease.

The dehydrogenase activity ranged between 1.6 and 4.3 mg triphenylformazane (TPF) kg^{-1} h^{-1} (Figure 5D) and decreased in the order pastures > organic agricultural fields > conventional greenhouses > conventional agricultural fields > fallows > references > organic greenhouses. The dehydrogenase activity of the pasture soils was significantly ($P < 0.05$) higher than that of soils of the conventionally managed agricultural fields, organic greenhouses, fallows and reference. Additionally, the organically managed greenhouse soils had a significantly ($P < 0.05$) lower dehydrogenase activity than those of the conventionally managed greenhouses and organically managed agricultural fields. The dehydrogenase activity of the fallow and reference soils did not differ from those of cultivated soils. Soils of the organically managed agricultural fields had a higher dehydrogenase activity than those of the greenhouses but no differences could be detected between the different management systems. Kandeler *et al*. (1996) reported a dehydrogenase activity of 3.7, 4.0 and 9.5 mg TPF kg^{-1} h^{-1} in a Dystric Lithosol, an Eutric Cambisol and a Calcaric Phaeozem, respectively, while Montero *et al*. (2004) found a dehydrogenase activity between 1.4 and 4.3 mg TPF kg^{-1} h^{-1} in non-tilled Entic Haplustolls and Vertic Argiudolls in three villages in Argentina, which is well in the range as the reported values.

The urease activity ranged between 31.8 and 58.7 mg ammonium (NH_4^+) kg^{-1} h^{-1} (Figure 5E) and decreased in the order fallows > conventional agricultural fields > references > pastures > conventional greenhouses > organic agricultural fields > organic greenhouses. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. The urease activity was tendentially higher in the conventionally managed soils in comparison to the organically managed ones, while the greenhouse soils exhibited lower values than those of the agricultural fields. The urease activity of the fallow, pasture and reference soils did not differ from those of the cultivated ones. Conti *et al*. (1998) reported an urease activity between 22 and 28 mg NH_4^+ kg^{-1} h^{-1} of a silty-loamy Typic Argiudoll of the Argentinean Pampa in the province of Cordoba, Argentina, while de la Paz Jimenez *et al*. (2002) found an urease activity between 5.4 and 33.6 mg NH_4^+ kg^{-1} h^{-1} in Vertic Argiudolls of forestal, pastural and agricultural managements located at La Paz in Entre Rios Province, Argentina. Both investigations exhibited an urease activity being at the lower end of the reported values.

Summarising, the reported ranges of individual enzyme activities can be considered as typical for soils under agricultural use. In general, the highest enzyme activities were found in soils of pastures and the reference. With respect to the differentiation of various land use systems, only the dehydrogenase activity exhibited significant ($P < 0.05$) differences between soils of conventional and organic managements and between those of indoor and outdoor cultivations.

3.1.3.2 Respiratory parameters

The basal respiration rate ranged between 2.7 and 6.1 mg carbon dioxide (CO_2) kg^{-1} h^{-1} (Figure 6A) and decreased in the order pastures > references > fallows > conventional agricultural fields > organic greenhouses > organic agricultural fields > conventional greenhouses. The pasture and reference soils exhibited a significantly ($P < 0.05$) higher basal respiration rate than those of all other land use systems, which did not show any trends between different management systems or indoor and outdoor cultivation. Emmerling and Udelhoven (2002) analysed a basal respiration rate of 1.6 and 3.0 mg CO_2 kg^{-1} h^{-1} in arable and grassland soils (Cambisols, Luvisols, Fluvisols and Stagnosols), respectively, while Svensson and Pell (2001) determined a rate between 0.4 – 1.5 mg CO_2 kg^{-1} h^{-1} in differently cropped systems of Haplic Phaeozems and Eutric Cambisols in southern Sweden. Teklay *et al*. (2006) found a basal respiration rate of 1.4 mg CO_2 kg^{-1} h^{-1} in Mollisols of agricultural fields in Wondo Genet, Ethiopia.

The substrate-induced respiration rate ranged between 50.6 and 92.7 mg CO_2 kg^{-1} h^{-1} (Figure 6B) and decreased in the order conventional greenhouses > organic greenhouses > pastures > organic agricultural fields > references > conventional agricultural fields > fallows. Soils of the conventionally managed agricultural fields and fallows exhibited significantly ($P < 0.05$) lower values than those of the two greenhouse cultivations and the pasture soils. Furthermore, the substrate-induced respiration rate of the conventional greenhouse soils was significantly ($P < 0.05$) higher than that of the organically managed agricultural field soils. The greenhouse soils exhibited a tendentially higher substrate-induced respiration rate in comparison to that of the agricultural field soils, while no differences with respect to the management systems were obvious. The fallow soils exhibited similarly low values as those of the conventional agricultural fields. Svensson and Pell (2001) found a substrate-induced

respiration rate between 6.9 and 17.1 mg CO_2 kg^{-1} h^{-1} in differently cropped systems of Haplic Phaeozems and Eutric Cambisols in southern Sweden, while Templer *et al*. (2005) analysed a rate of 48 mg CO_2 kg^{-1} h^{-1} in agricultural soils of the Los Haitises region of the Dominican Republic. The mean rate by Teklay *et al*. (2006) ranged from 10.0 to 18.0 mg CO_2 kg^{-1} h^{-1} in Mollisols of agricultural fields in Wondo Genet, Ethiopia, depending on nutrient supply. Hence, the reported values were up to ten times higher than those of comparable soils given in literature, which was eventually caused by methodological differences.

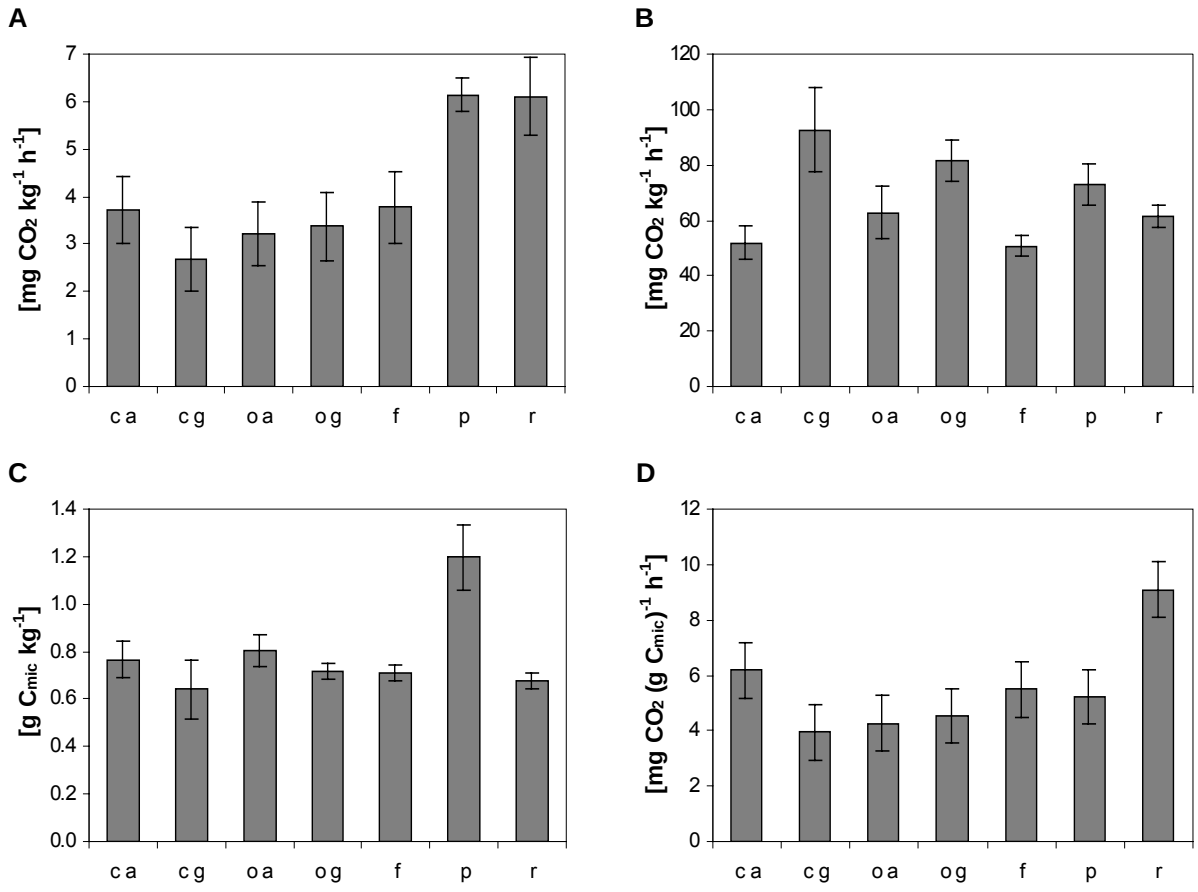

Figure 6: Respiratory parameters in soils of the land use systems under study with standard error bars; c: conventional, o: organic, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference; **A**: basal respiration, **B**: substrate-induced respiration, **C**: microbial biomass, **D**: metabolic quotient.

The microbial biomass carbon content (C_{mic}) ranged between 0.64 and 1.20 g C_{mic} kg^{-1} (Figure 6C) and decreased in the order pastures > organic agricultural fields > conventional agricultural fields > organic greenhouses > fallows > references > conventional greenhouses. The pasture soils exhibited a significantly ($P < 0.05$) higher content than those of all other

land use systems. A tendentially higher content was found in soils of the agricultural fields in comparison to the greenhouse soils, while no differences were detected between soils of the organic and conventional managements. The content of the fallow and reference soils did not differ from that of all cultivated soils. Alvarez *et al.* (1998) found a microbial biomass content of 0.2 – 0.5 and 1.5 g C_{mic} kg^{-1} in soils of agricultural fields and pastures, respectively, in Typic Argiudolls of a study area of the INTA in Pergamino, Argentina. Emmerling and Udelhoven (2002) reported 0.30 and 0.47 g C_{mic} kg^{-1} in arable and grassland soils (Cambisols, Luvisols, Fluvisols and Stagnosols), respectively, while Mishra *et al*. (2005) determined a biomass content between 1.0 and 3.5 g C_{mic} kg^{-1} in pastural Inceptisols and Alfisols of the USA. Templer *et al*. (2005) analysed a biomass content of 1.7 g C_{mic} kg^{-1} in agricultural soils of the Los Haitises region of the Dominican Republic. Therefore, the present study confirmed a higher biomass content in pastures compared to agricultural fields.

The metabolic quotient ranged between 4.0 and 9.1 mg CO_2 (g C_{mic})$^{-1}$ h^{-1} (Figure 6D) and decreased in the order references > conventional agricultural fields > fallows > pastures > organic greenhouses > organic agricultural fields > conventional greenhouses. The values of the reference soils were significantly ($P < 0.05$) higher than of those of all other land use systems. The metabolic quotient is a measure of how effective microorganisms are. Hence, higher values indicate lower efficiency in microbial transformation activity and in conserving carbon resources in the reference than in agriculturally used soils. Comparable to the basal respiration no trends were found towards the management systems or the indoor and outdoor cultivations.

Corroborating the results of the enzyme activities, the pasture and reference soils showed the highest values of the respiratory parameters, while except for the substrate-induced respiration no significant ($P > 0.05$) differences between the soils of conventional and organic managements and between indoor and outdoor cultivations could be detected. The reported respiratory parameter values (except for substrate-induced respiration) were comparable to those of other studies and can thus be considered as typical values for agricultural soils.

3.1.3.3 Nitrogen parameters

The net nitrogen mineralisation rate ranged between 1.0 and 2.8 mg N_{min} kg^{-1} d^{-1} (Figure 7A) and decreased in the order references > conventional greenhouses > pastures >

conventional agricultural fields > organic agricultural fields > organic greenhouses > fallows. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. The conventionally cultivated soils exhibited a tendentially higher net nitrogen mineralisation rate than the organically cultivated ones. The highest rate of the reference soils indicated reductions in supply of naturally plant-available nitrogen in all agriculturally used soils, while the net nitrogen mineralisation rate of the fallow and pasture soils did not differ from the cultivated ones. Mishra *et al*. (2005) determined a net nitrogen mineralisation rate between 0.4 and 5.9 mg N_{min} kg^{-1} d^{-1} in pastural Inceptisols and Alfisols of the USA, while Templer *et al*. (2005) found a rate of 0.9 mg N_{min} kg^{-1} d^{-1} in agricultural soils of the Los Haitises region of the Dominican Republic. Both studies showed values for the net nitrogen mineralisation in the same order of magnitude as the reported ones.

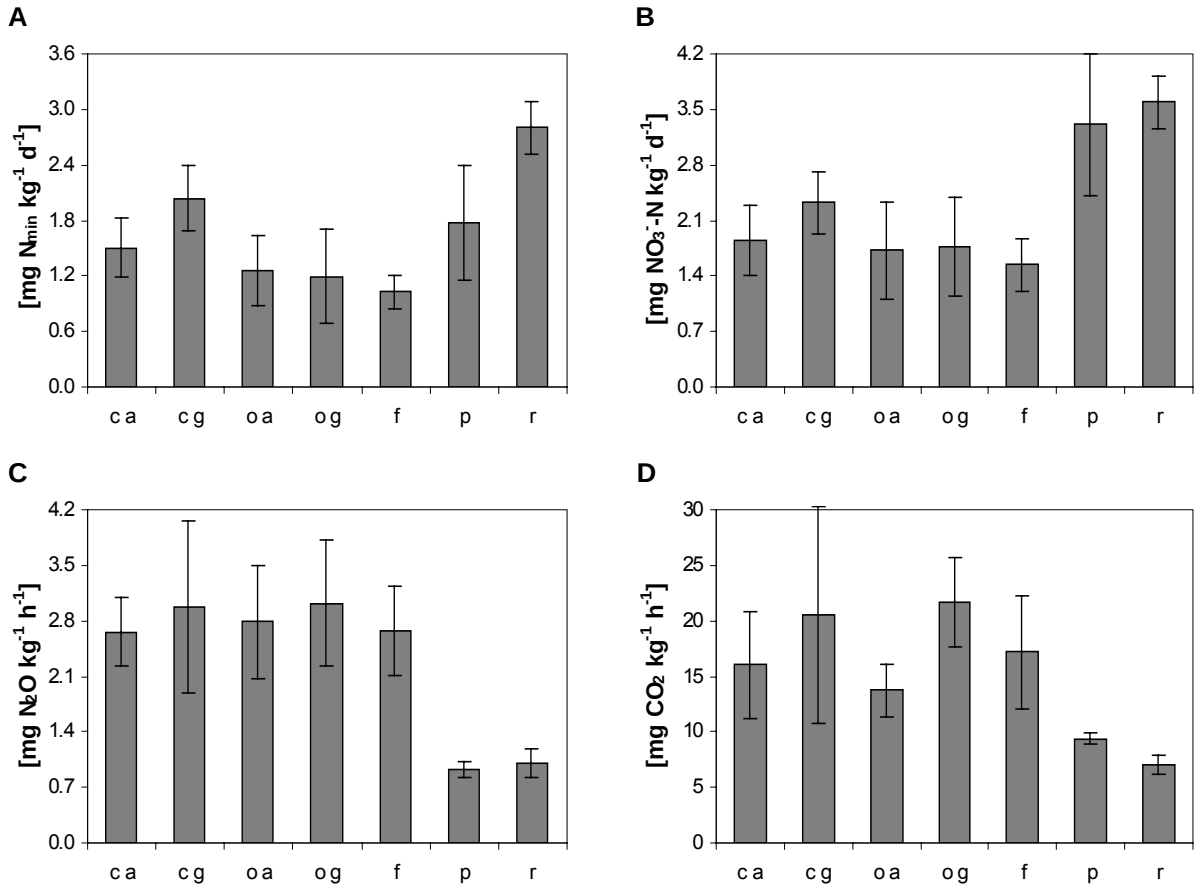

Figure 7: Nitrogen parameters in soils of the land use systems under study with standard error bars; o: organic, c: conventional, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference; **A**: net nitrogen mineralisation, **B**: net nitrification, **C** and **D**: N_2O and CO_2 emission at the potential denitrification, respectively.

The net nitrification rate ranged between 1.7 and 3.6 mg NO_3^--N kg^{-1} d^{-1} (Figure 7B) and decreased in the order references > pastures > conventional greenhouses > conventional agricultural fields > organic greenhouses > organic agricultural fields > fallows. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. With the exception of a distinctly higher net nitrification rate in the pasture soils these results corresponded to those of the net nitrogen mineralisation. Templer *et al*. (2005) analysed a net nitrification rate of 0.9 mg NO_3^--N kg^{-1} d^{-1} in agricultural soils of the Los Haitises region of the Dominican Republic.

The potential denitrification rate ranged between 0.9 and 3.0 mg N_2O kg^{-1} h^{-1} (Figure 7C) and decreased in the order organic greenhouses > conventional greenhouses > organic agricultural fields > fallows > conventional agricultural fields > references > pastures. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. A tendentially higher rate was found in soils of the greenhouses compared to those of the agricultural fields, while no differences in the management systems were detected. The pasture and reference soils showed much lower values than the other ones possibly due to the lower pH values (Table 3, page 37) since the optimum for the potential denitrification is known being pH 7– 8 (Šimek and Hopkins, 1999). Templer *et al*. (2005) analysed a potential denitrification rate of 1.0 mg N_2O kg^{-1} d^{-1} in agricultural soils of the Los Haitises region of the Dominican Republic being in the same order of magnitude as the reported values, while Svensson and Pell (2001) found a lower rate between 0.1 – 0.6 mg N_2O kg^{-1} h^{-1} in differently cropped systems of Haplic Phaeozems and Eutric Cambisols in southern Sweden.

The CO_2 emission rate of the potential denitrification under anaerobic condition ranged between 7.0 and 21.6 mg CO_2 kg^{-1} h^{-1} (Figure 7D) and decreased in the order organic greenhouses > conventional greenhouses > fallows > conventional agricultural fields > organic agricultural fields > pastures > references. However, because of the high standard errors no significant differences ($P > 0.05$) could be detected. The trend corresponded to that of the N_2O emission rate of the potential denitrification because during anaerobic respiration N_2O and CO_2 are released simultaneously from the soil.

The potential denitrification is the only soil microbial functional parameter, which exhibited the lowest values for the pasture and reference soils, while these soils showed the highest values for the net nitrogen mineralisation and net nitrification. Furthermore, no

significant differences ($P > 0.05$) were observed between soils of the organic and conventional management systems and between indoor and outdoor cultivations. However, as the values were similar to those of other investigations about agricultural soils, they seem to be typical for soils under these land use systems.

3.1.3.4 Principal component analysis

As shown above, by means of individual soil microbial community functional parameters it was not possible to distinguish between soils of different land use systems, in particular between soils of different management systems as well as indoor and outdoor cultivations. Hence, a principal component analysis (PCA) was applied in order to pool correlating data into independent components. For the analysed parameter values a Kaiser-Meyer-Olkin-measure of sampling adequacy of 0.573 was computed, which indicated that a PCA was useful for the data set, while a value lower than 0.0005 calculated from the Bartlett's Test of Sphericity exhibited that significant relationships among the analysed parameters existed and that the data set was suitable for a PCA (Backhaus and Erichson, 2003). Four principal components explaining 75.6 % of the total variance were extracted via PCA (Table 8).

Table 8: Varimax rotated (after Kaiser normalisation) component matrix of the principal component analysis of all investigated soil microbial functional parameters. Each component combines variables with the highest factor loadings (bold) in a column.

Microbial functional parameters	Component 1	Component 2	Component 3	Component 4	Interpretation
Microbial biomass	**0.91**	0.10	0.09	0.00	
Arylsulfatase activity	**0.80**	0.16	-0.04	0.19	Microbial
Acid phosphatase activity	**0.64**	0.15	-0.62	0.17	capacity
Dehydrogenase activity	**0.55**	0.23	-0.43	-0.16	
Net nitrogen mineralisation	0.07	**0.85**	-0.03	0.13	
Net nitrification	0.35	**0.82**	-0.11	0.26	Mineralisation
Substrate-induced respiration	0.10	**0.74**	0.32	-0.16	activity
Cellulase activity	0.17	**0.61**	-0.27	0.35	
Potential denitrification (N_2O)	-0.01	0.03	**0.87**	-0.27	Nitrogen
Potential denitrification (CO_2)	-0.18	0.18	**0.82**	-0.02	transformation
Urease activity	0.26	-0.34	**0.55**	-0.21	potential
Metabolic quotient	-0.06	0.14	-0.20	**0.93**	Metabolic
Basal respiration	0.54	0.22	-0.14	**0.77**	activity

Besides the microbial biomass carbon the first component explaining 21.0 % of the total variance (microbial capacity) included arylsulfatase, acid phosphatase (not highly specific because of similarly high correlation coefficients in two components) and dehydrogenase activities. This result was concordant with that of Taylor *et al*. (2002) who examined strong correlations between these parameters in comparison of enzyme activities using various techniques. This indicated that the bigger the microbial biomass the more enzymes are released into the soil in order to degrade large molecules of the soil organic matter and the higher is the catabolic activity regarding the dehydrogenase activity. The second component (mineralisation activity) explaining 20.4 % of the total variance was composed of nitrogen (net nitrogen mineralisation and net nitrification) and carbon (substrate-induced respiration and cellulase activity) mineralisation parameters. In ecosystems, in which easily degradable organic matter such as organic manure is incorporated the activity of hydrolytic enzymes like cellulases is stimulated whereby glucose is produced. This glucose release increases the substrate-induced respiration that enhances the mineralisation activity of the soil microbes. The third component (nitrogen transformation potential) explaining 19.7 % of the total variance contained anaerobic N_2O and CO_2 emission rates of the potential denitrification and urease activity. Particularly, the N_2O and CO_2 emissions correlated since nitrate is used as oxygen source during the anaerobic respiration and is degraded simultaneously to N_2O and CO_2. The fourth component (metabolic activity) explaining 14.5 % of the total variance consisted of the metabolic quotient and basal respiration, which are both indicators of turn-over and instability of the microbial biomass (Wardle *et al*., 1999).

3.1.3.5 Hierarchical cluster analysis

Subsequent to the principal component analysis (PCA) a first discriminant analysis was performed calculated via the factor scores of the PCA in order to distinguish between soils of the seven diverse land use systems. In this analysis strong similarities between conventional agricultural fields and fallows (data not shown) were ascertained. As already mentioned above, all fallow sites were previously conventionally cultivated. Hence, a differentiation of soils with the aid of soil microbial functional parameters possibly only works in medium or long term land uses and is less suitable for short term changes. Consequently, these two land use systems should show similarities with regard to these investigated parameters. In order

to clarify this hypothesis, a hierarchical cluster analysis was conducted demonstrating that soils of conventionally managed fields and fallows are very similar (Figure 8). Furthermore, similarities between soils of conventionally and organically managed agricultural fields and between soils of conventionally and organically managed greenhouses could be observed. Therefore, not the cultivation systems, but on a small scale climatic or other factors resulting from varieties of indoor or outdoor cultivation were more responsible for differences in soil microbial community functional parameters within agricultural managements. The reference and pasture soils were distinctly separated from these agricultural management systems and formed own clusters in each case (Figure 8).

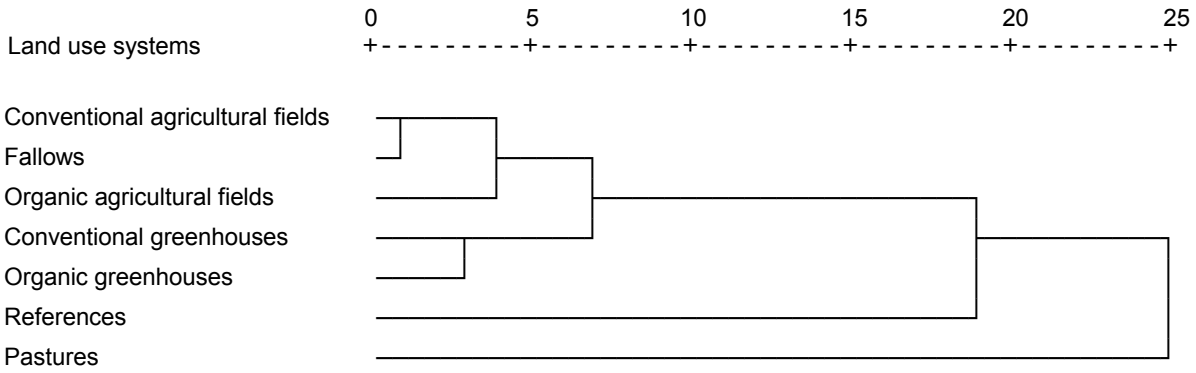

Figure 8: Dendrogram of the hierarchical cluster analysis of the land use system soils under study.

3.1.3.6 Discriminant analysis

By reason of the results obtained by the cluster analysis, a second discriminant analysis was performed. Soils of the same land use were expected being in the same discriminant group (Table 9). The two land use systems conventional agricultural fields and fallows were combined into group A (conventional agricultural field). 96.9 % of the total variance of all group centroids were described by the first (78.2 %) and the second (18.7 %) canonical discriminant function and correlation coefficients close to 1 (0.965 and 0.873) indicated strong correlations between discriminant scores and groups. Very low Wilks' Lambda values close to 0 (0.011 and 0.153) showed that the group means were different, which was confirmed by a high significance greater than 99.5 %. The third and fourth canonical discriminant function explaining 2.9 and 0.2 % of the total variance, respectively, were insignificant. In Figure 9 the first two resulting canonical discriminant functions are plotted and it is visible that a differentiation among groups of specific land use systems was possible.

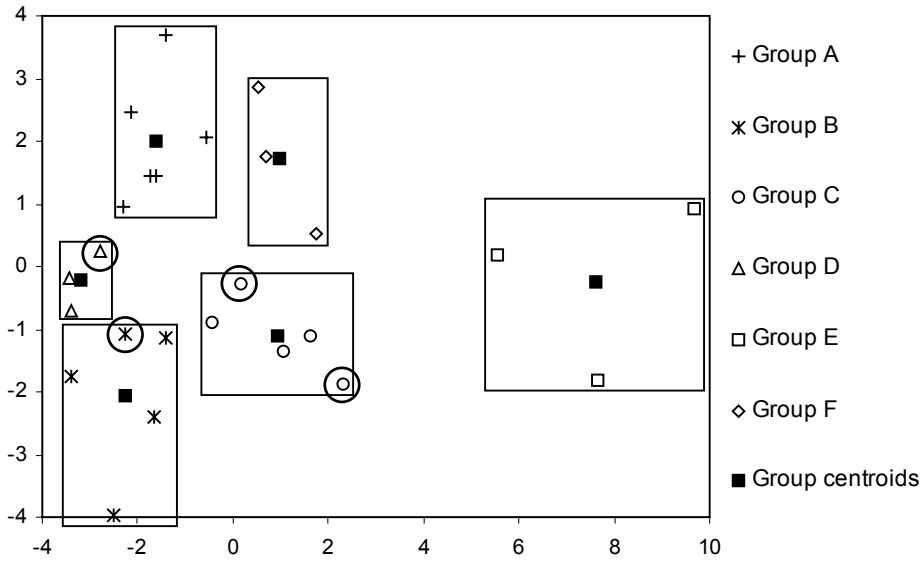

Figure 9: Plot of the values of canonical discriminant function 1 and 2 (x- and y- axis, respectively) at six classification items. Framed sampling sites could not be correctly allocated (see Table 9).

According to the actual groupings of soils of the different land use systems of the discriminant analysis (Table 9) a definite separation between soils of pastures, references and conventional agricultural fields including fallows compared to the other soils could be found because these land use systems were allocated into the expected groups. The organic greenhouse of site 3 and the organic agricultural field of site 2 were allocated to group C (organic agricultural field) and group D (organic greenhouse), respectively. This could be explained by a change of indoor and outdoor cultivation because farmers around Buenos Aires often cultivate in greenhouses at first and then remove them and use the field outdoor and vice versa. The conventional agricultural field of site 4 was allocated to group C (organic agricultural field). This site was an agricultural research area, which belonged to the INTA in La Plata. Due to previous investigations this site was formerly organically managed and only recently by reasons of other concerns its land use has been changed into conventional management. Ultimately, the fallow of site 1 was conventionally cultivated in former times, however, it was used as a greenhouse site. Consequently, the fallow soils were not long enough under this land use and thus, they still exhibited the same soil microbial functional properties like soils of conventionally managed fields, which they were before. Therefore, the reported results of the statistical analysis clearly showed that this approach could serve as a test procedure to differentiate between soils of different medium- to long-term agricultural land use practices when compared to anthropogenically unaffected sites.

Table 9: Allocation of soils of six different land use systems into groups via discriminant analysis (conventional agricultural fields combined with fallows in group A). Bold sampling sites could not be correctly allocated.

Discriminant groups	Land use systems	Expected groupings	Actual groupings
Group A	Conventional agricultural field (ca)	ca 1, ca 2, ca 3, ca 4 f 1, f 2, f 3, f 4	ca 1, ca 2, ca 3, f 2, f 3, f 4
Group B	Conventional greenhouse (cg)	cg 1, cg 2, cg 3, cg 4	cg 1, cg 2, cg 3, cg 4, **f1**
Group C	Organic agricultural field (oa)	oa 1, oa 2, oa 3, oa 4	oa 1, oa 3, oa 4, **og 3**, **ca 4**
Group D	Organic greenhouse (og)	og 1, og 2, og 3	og 1, og 2, **oa 2**
Group E	Pasture (p)	p 1, p 2, p 3	p 1, p 2, p 3
Group F	Reference (r)	r 1, r 2, r 3	r 1, r 2, r 3

1 – 4: sampling sites.

3.1.3.7 Ecological significance

The ecological significance of the investigated parameters among the different land use systems can tendentially be estimated in Figure 10, in which parts A, B, C and D exhibit the factor scores of the principal components (PC, Table 8, page 54): PC 1 (Microbial capacity: soil microbial biomass carbon, arylsulfatase, acid phosphatase and dehydrogenase activities), PC 2 (Mineralisation activity: substrate-induced respiration, cellulase activity, net nitrogen mineralisation and net nitrification), PC 3 (Nitrogen transformation potential: potential denitrification and urease activity) and PC 4 (Metabolic activity: basal respiration and metabolic quotient), respectively. According to this, the soils of conventional agricultural fields and fallows (group A of the discriminant analysis) showed medium levels of PC 1 and 3, the lowest levels of PC 2 and high levels of PC 4. The soils of conventional greenhouses (group B of the discriminant analysis) were characterised by the lowest levels of PC 1 and 4, medium levels of PC 3 and the highest levels of PC 2. The soils of organic agricultural fields (group C of the discriminant analysis) exhibited medium levels of all PC, while soils of the organic greenhouses (group D of the discriminant analysis) showed low levels of PC 1, medium levels of PC 2 and 4 and the highest levels of PC 3. The pasture soils (group E of the discriminant analysis) were characterised by the significantly ($P < 0.05$) highest levels of PC 1, medium levels of PC 2 and 4 and the lowest levels of PC 3. The reference soils (group F of the discriminant analysis) had the significantly ($P < 0.05$) highest levels of PC 4, high levels of PC 1 and 2 and low levels of PC 3.

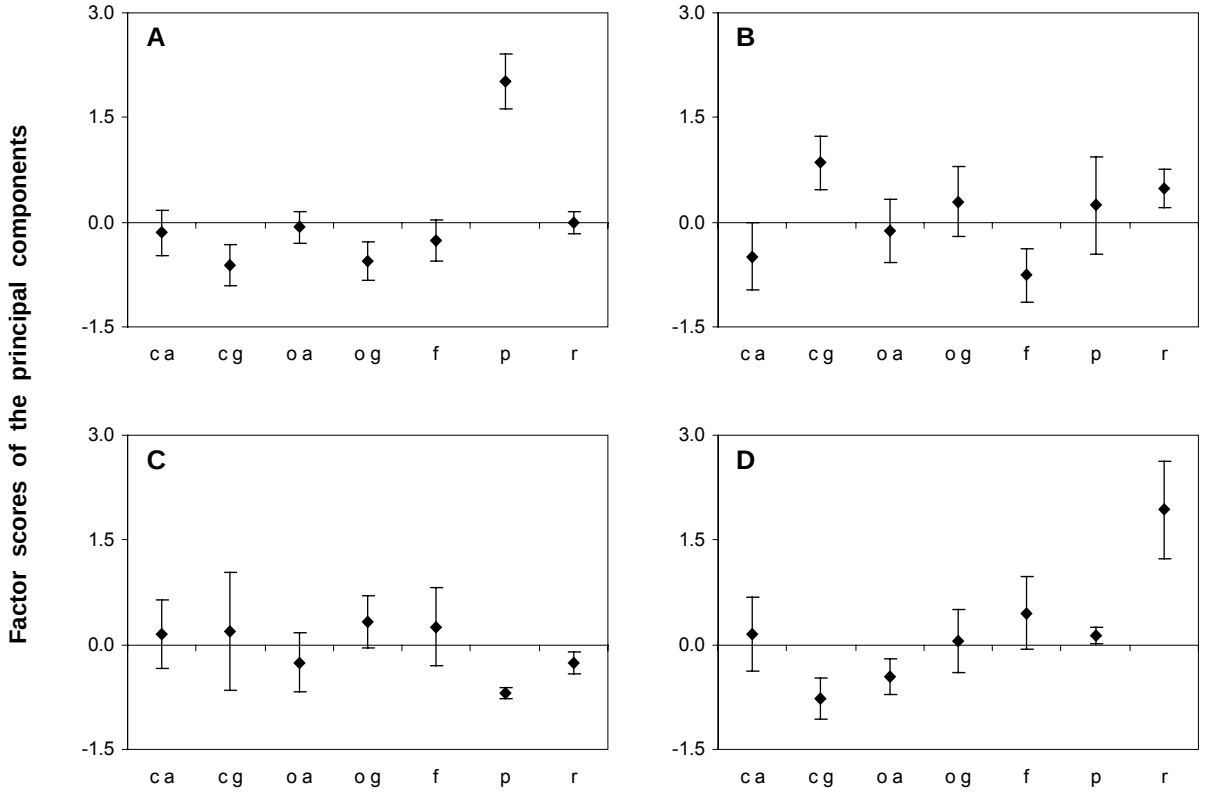

Figure 10: Factor scores of all four principal components (PC) of the land use systems under study with standard error bars; c: conventional, o: organic, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference; **A**: PC 1 (Microbial capacity: microbial biomass carbon, acid phosphatase, arylsulfatase and dehydrogenase activities), **B**: PC 2 (Mineralisation activity: substrate-induced respiration, cellulase activity, net nitrogen mineralisation and net nitrification), **C**: PC 3 (Nitrogen transformation potential: potential denitrification and urease activity) and **D**: PC 4 (Metabolic activity: basal respiration and metabolic quotient).

Therefore, in comparison to the anthropogenically unaffected soils it was obvious that any change of soil management into agricultural land use led to a strong reduction of the metabolic activity (basal respiration and metabolic quotient), while the remaining components were affected only slightly. Mainly pasture soils showed an enhanced microbial capacity (microbial biomass carbon and arylsulfatase, dehydrogenase and acid phosphatase activities) and a reduced nitrogen transformation potential (potential denitrification and urease activity). The soils of greenhouse managements exhibited only a tendentially reduced microbial capacity (microbial biomass carbon and arylsulfatase, dehydrogenase and acid phosphatase activities) and a slightly enhanced mineralisation activity (substrate-induced respiration, cellulase activity, net nitrogen mineralisation and net nitrification), while among conventional and organic management systems no differences were found.

3.1.4 Structural parameters

3.1.4.1 Microbial biomass

The sum of all determined phospholipid fatty acid (PLFA) concentrations is a measure of a viable microbial biomass, since phospholipids are readily degraded after cell death (Zelles, 1997) and thus, these biomass values can be compared for every land use system (Figure 11). Joergensen and Emmerling (2006) recently have published a conversion factor of 5.8 ± 2.3 of total PLFA contents (μmol kg^{-1}) into microbial biomass carbon (mg kg^{-1}), while in the present investigation a conversion factor of 10.4 ± 2.6 (N = 69) was calculated between total PLFA content and microbial biomass carbon content (calculated via substrate-induced respiration, 3.1.3.2, page 50). However, conversion factors are only reasonable for particularly defined PLFA to be analysed and converted into microbial biomass. Otherwise, diversely calculated biomass data are not comparable. Therefore, the results are further on presented as total PLFA concentration instead of microbial biomass, which decreased in the soils of different land use systems in the order references (100 % ± 15.6 %) > pastures (81.5 ± 7.8 %) > organic agricultural fields (72.6 ± 7.8 %) > conventional agricultural fields (65.1 ± 5.2 %) > organic greenhouses (62.1 ± 6.2 %) > conventional greenhouses (61.5 ± 9.1 %) > fallows (55.0 ± 4.3 %). The differences between the reference soils and those of every other land use system besides the pastures and organic agricultural fields were significant ($P < 0.05$). Hence, any change to agricultural land use leads to a reduction of soil microbial biomass. Additionally, the second highest biomass content in the pasture soils was significantly ($P < 0.05$) higher than that of the lowest of the fallow soils. This means that pasture soils were able to maintain microbial biomass due to nutrient inputs through animal manure but the fallow soils exhibited no recovery from agricultural land use. Within the various cultivation systems, no significant ($P > 0.05$) differences in microbial biomass content were found. These results are comparable to those of Waldrop *et al*. (2000) who showed that the microbial biomass content in various cultivated soils were not significantly different. Also Wander *et al*. (1995) and Petersen *et al*. (1997) could not find significant differences in microbial biomass content between differently cultivated Alfisols and Inceptisols in Pennsylvania and Typic Agrudalfs developed on glacial deposits, respectively. In comparison of indoor and outdoor cultivations of the same management systems it is visible that the latter tendentially exhibited a higher microbial

biomass content, which corresponded to the results of Ibekwe and Kennedy (1998) who detected a lower content in greenhouse Mollisols than on agricultural field ones.

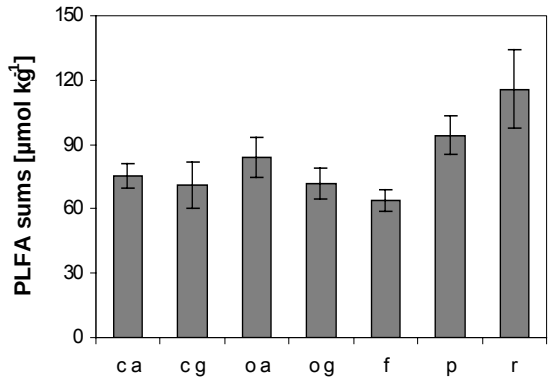

Figure 11: Total PLFA content in soils of the land use systems under study with standard error bars; c: conventional, o: organic, a: agricultural field, g: greenhouse, f: fallow, p: pasture, r: reference.

The total PLFA content correlated highly significantly ($P < 0.01$) with total organic carbon (TOC) and total organic nitrogen (TON) contents (Figure 12a) indicating a close connection between nutrient concentrations and the amount of the microbial biomass. In addition, the total PLFA content showed highly significant ($P < 0.01$) correlations to soil microbial function parameters such as acid phosphatase and arylsulfatase activity, basal and substrate-induced respiration, soil microbial biomass (calculated using the substrate-induced respiration) and net nitrification (Figure 12b). The correlation with soil microbial biomass corresponded to the

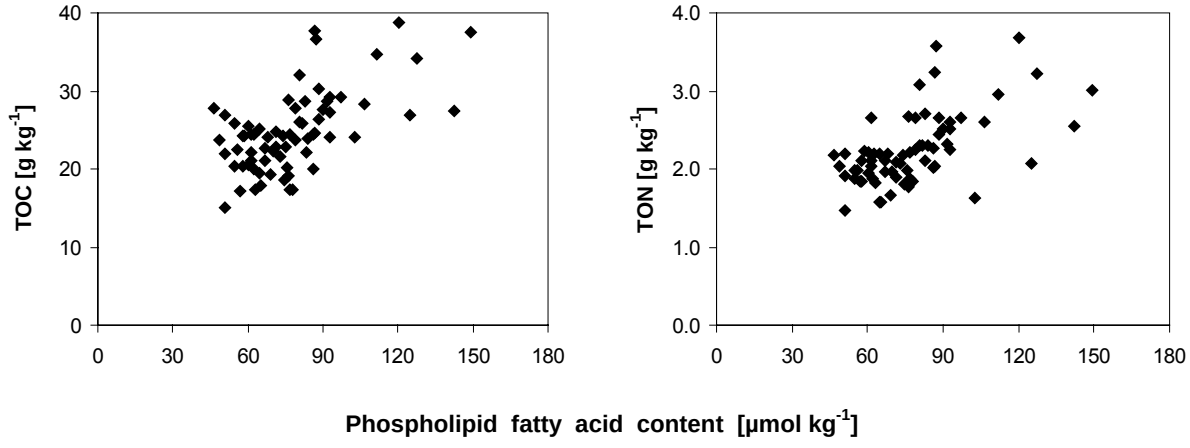

Figure 12a: Correlation plots ($P < 0.01$, $N = 69$) of total PLFA content with total organic carbon (TOC; $R = 0.557$) and total organic nitrogen (TON; $R = 0.562$) contents.

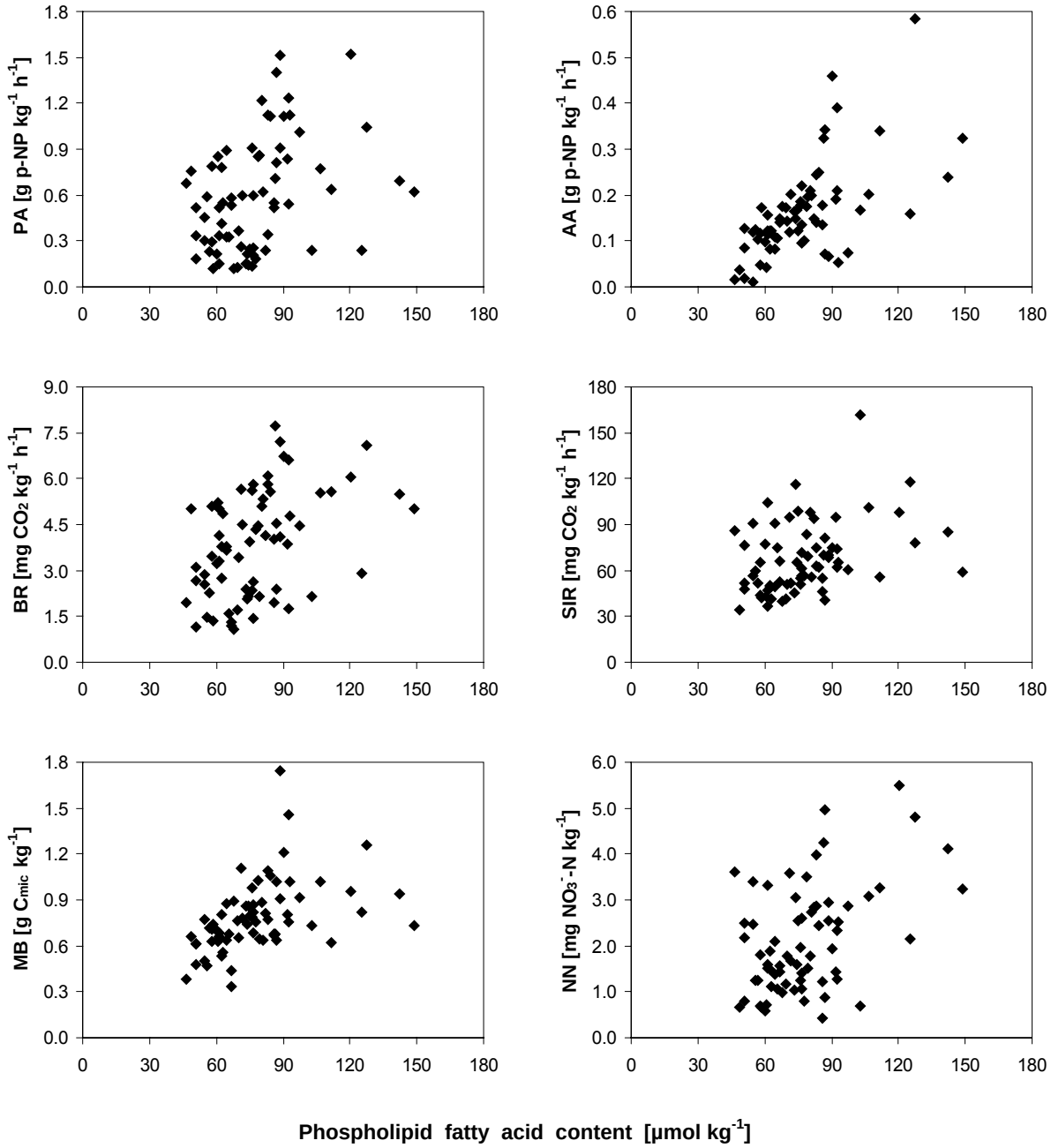

Figure 12b: Correlation plots (P < 0.01, N = 69) of total PLFA content with soil microbial functional parameters such as acid phosphatase activity (PA; R = 0.429), arylsulfatase activity (AA; R = 0.636), basal respiration (BR; R = 0.441), substrate-induced respiration (SIR; R = 0.375), microbial biomass (MB; R = 0.587) and net nitrification (NN; R = 0.369).

results as reported for Typic Agrudalfs (Petersen *et al.,* 1997), for red soils of south-western China (Yao *et al.,* 2000) and for forest soils of northern Germany (Bååth and Anderson, 2003) but no correlation with the pH value could be found in contrast to the latter investigation.

3.1.4.2 Principal component analysis

Individual PLFA cannot be used to represent specific species both because an individual bacterial or fungal species can contain numerous fatty acids and because the same fatty acids occur in many different organisms. However, with the aid of a principle component analysis (PCA) correlating PLFA were pooled into independent principal components, which then could be allocated to different microbial taxonomic groups according to literature (see below). For this purpose, relative abundances of every PLFA were calculated, each PLFA being used as variable in the PCA. A Kaiser-Meyer-Olkin-measure of sampling adequacy of 0.687 was computed, which indicated that a PCA was useful for the data set, while a value lower than 0.0005 calculated from the Bartlett's Test of Sphericity exhibited that significant relationships among the variables existed and that the data were suitable for PCA (Backhaus and Erichson, 2003). Four principal components explaining 77.8 % of the total variance were extracted via PCA (Table 10). Allocations of the determined PLFA to particular microbial groups according to the PCA are summarised in Table 11 and are discussed below.

Table 10: Varimax rotated (after Kaiser normalisation) component matrix of the principal component analysis of individual PLFA. Each component combines variables with the highest factor loadings (bold) in a column. PLFA 10Me16:0 was unspecific because it showed high factor loadings in two components (italic and underlined).

PLFA	Component 1	Component 2	Component 3	Component 4
10Me17:0	**0.91**	-0.09	0.03	0.15
10Me18:0	**0.84**	-0.22	0.18	-0.03
cy17:0	**0.78**	0.48	0.17	0.20
a17:0	**0.74**	-0.27	0.44	0.05
20:4ω6	**0.66**	0.26	-0.26	-0.18
i17:0	**0.63**	-0.33	0.14	0.46
18:1ω7c	**-0.59**	**0.47**	-0.34	-0.08
16:1ω5c	0.09	**0.89**	-0.00	-0.07
16:1ω7c	-0.32	**0.87**	-0.12	0.05
10Me16:0	0.01	*-0.64*	0.06	*0.60*
a15:0	0.09	-0.00	**0.92**	0.00
i15:0	0.03	0.01	**0.80**	0.33
i16:0	0.47	-0.42	**0.64**	0.18
18:1ω9c	-0.01	-0.11	-0.17	**-0.89**
18:2ω6,9	-0.38	0.31	-0.32	**-0.47**

Table 11: Allocation of PLFA to microbial groups according to the principal component analysis.

Gram +, anaerobic b.	Actino-mycetes	Protozoa	Gram - bacteria	Gram +, aerobic b.	Fungi	Not representative	Unspecific PLFA
cy17:0 [A,B]	10Me17:0 [A]	20:4ω6 [D]	16:1ω5c [A]	a15:0 [A,C]	18:1ω9c [E,F]	10Me16:0	14:0 [A]
a17:0 [A,C]	10Me18:0 [A]		16:1ω7c [A,F]	i15:0 [A,C]	18:2ω6,9 [E,F]		15:0 [A]
i17:0 [A,C]			18:1ω7c [A,F]	i16:0 [A,C]			16:0 [A]
[A] = Zelles, 1999; [B] = Burke *et al*., 2003; [C] = Steinberger *et al*., 1999							17:0 [A]
[D] = Cavigelli *et al*., 1995; [E] = Ruess *et al*., 2002; [F] = Treonis *et al*., 2004							18:0 [A]

b.: bacteria.

The PLFA cy17:0, a17:0, i17:0, 10Me17:0, 10Me18:0 and 20:4ω6 were combined in the first component explaining 28.9 % of the total variance. Within this component three microbial groups were in close connection to each other. The PLFA cy17:0 is a biomarker for both Gram positive anaerobic and Gram negative bacteria (Burke *et al*., 2003; Zelles, 1999). Because of the fact that the methyl-branched PLFA a17:0 and i17:0, which are biomarkers for Gram positive bacteria (Zelles, 1999; Steinberger *et al*., 1999), were also allocated into this component, these three PLFA were considered as biomarkers for Gram positive, anaerobic bacteria. Furthermore, the PLFA 10Me17:0 and 10Me18:0 – generally known as biomarkers for actinomycetes (Zelles, 1999) – and the PLFA 20:4ω6 representing the protozoa (Cavigelli *et al*., 1995) also showed high factor loadings in the first component. Consequently, both microbial groups also being able to live under anaerobic conditions (Amador *et al*., 2006) exhibited strong correlations to the above-mentioned taxonomic group for the investigated data set.

The non-branched, mono-unsaturated PLFA 16:1ω5c and 16:1ω7c in the second principal component explaining 19.9 % of the total variance are biomarkers for Gram negative bacteria (Zelles, 1999; Treonis *et al*., 2004). The PLFA 18:1ω7c showed a high negative factor loading in the first component, which indicated an opposite trend than the Gram positive, anaerobic bacteria, actinomycetes and protozoa. This PLFA also had a quite high factor loading in the second component. According to Treonis *et al*. (2004) the PLFA 18:1ω7c also stands for Gram negative bacteria and thus, it was included into the second component. However, by reason of the high factor loadings in the two principal components it was not very sensitive for this microbial group.

The third component explaining 16.7 % of the total variance contained anteiso- and iso-branched PLFA a15:0, i15:0 and i16:0, which are known as biomarkers for Gram positive bacteria (Zelles, 1999; Steinberger *et al*., 1999). Therefore, this component combined Gram positive, aerobic bacteria since these PLFA did not correlate with those of the first, which has already included Gram positive, anaerobic bacteria.

Within the fourth component explaining 12.3 % of the total variance the PLFA 18:1ω9c and 18:2ω6,9 exhibited the highest negative factor loadings. These PLFA are typical for fungi (Treonis *et al*., 2004). Ruess *et al*. (2002) extracted these two PLFA at high concentration in membranes of 16 different taxonomic groups of fungi living in soil. Moreover, a significant positive correlation between PLFA 18:2ω6,9 and ergosterol was detected, which is known as a characteristic compound of fungal cell membranes (Bååth and Anderson, 2003).

The PLFA 10Me16:0 exhibited similar factor loadings in two principal components, while it showed an opposite trend than the remaining PLFA in both components. Therefore, this PLFA – generally known as biomarker for actinomycetes (Zelles, 1999) – was not considered being representative for any particular taxonomic group in this study because it could also be derived from other microorganisms such as sulfate reducing bacteria (Pelz *et al*., 2001; Chang *et al*., 2001), anaerobic ammonium oxidizing bacteria (Mills *et al*., 2006) and generally from all eubacteria (Wakeham *et al*., 2006).

In the following text, absolute PLFA contents and their percentage changes in soils of the different land use systems relative to the reference soils (Table 12) are discussed for every microbial taxonomic group.

Table 12: Percentage changes of absolute PLFA contents of the microbial taxonomic groups in soils of the land use systems under study relative to the reference soils with standard errors.

Land use systems	Gram +, anaerobic b.	Actino-mycetes	Protozoa	Gram - bacteria	Gram +, aerobic b.	Fungi
Conv. agr. field	64.4 ± 8.6	63.2 ± 8.4	86.0 ± 5.5	82.4 ± 4.8	52.3 ± 6.8	76.3 ± 4.7
Conv. greenh.	54.2 ± 3.6	50.1 ± 7.8	48.9 ± 16.4	64.7 ± 13.0	53.1 ± 7.4	63.0 ± 8.7
Org. agr. field	67.2 ± 6.7	58.8 ± 9.1	83.3 ± 12.6	95.8 ± 14.3	60.6 ± 8.6	84.3 ± 10.2
Org. greenh.	51.6 ± 4.4	44.4 ± 8.8	84.9 ± 26.8	71.3 ± 6.6	53.6 ± 7.8	76.9 ± 9.5
Fallow	50.1 ± 4.7	47.4 ± 9.0	73.0 ± 19.9	73.4 ± 9.5	46.1 ± 2.3	58.6 ± 11.4
Pasture	80.6 ± 11.8	87.8 ± 10.5	77.2 ± 17.6	86.7 ± 12.8	74.7 ± 13.3	89.8 ± 4.3
Reference	100.0 ± 19.1	100.0 ± 19.9	100.0 ± 11.8	100.0 ± 14.7	100.0 ± 20.4	100.0 ± 15.7

Conv.: conventional, org.: organic, agr.: agricultural, greenh.: greenhouse, b.: bacteria.

3.1.4.3 Individual microbial taxonomic groups

The PLFA content indicating Gram positive, anaerobic bacteria (Figure 13) decreased in the order references > pastures > organic agricultural fields > conventional agricultural fields > conventional greenhouses > organic greenhouses > fallows. The content of the reference soils was significantly ($P < 0.05$) higher than that in soils of the other land use systems besides the organic agricultural fields and the pastures, which showed significantly ($P < 0.05$) higher values than soils of the conventionally and organically managed greenhouses and the fallows. The PLFA content in soils of the organic agricultural fields was also significantly ($P < 0.05$) higher than in the fallow soils. Greenhouse cultivation apparently increased the effect that any agricultural land use decreased the microbial biomass. However, differences between the organically and conventionally cultivated systems could not be found. Gram positive, anaerobic bacteria reduced up to 50 % in fallow soils, while pasture soils showed the lowest decrease up to 20 %. The biomass of Gram positive, anaerobic bacteria exhibited highly significant ($P < 0.01$) correlations with TOC ($R = 0.544$) and TON ($R = 0.569$) contents. A highly significant ($P < 0.01$) negative correlation was found with the pH value ($R = – 0.339$).

The PLFA content indicating actinomycetes (Figure 13) decreased in the order references > pastures > conventional agricultural fields > organic agricultural fields > conventional greenhouses > fallows > organic greenhouses. The reference soils contained the significantly ($P < 0.05$) highest actinomycetes biomass compared to that in soils of the other land use systems besides the pastures, which showed significantly ($P < 0.05$) higher values than soils of the conventionally and organically managed greenhouses and fallows. Thus, the content of actinomycetes relative to the reference soils behaved similar in comparison to the Gram positive, anaerobic bacteria besides those of soils of the organic agricultural fields, which showed decreases up to 41 % compared to 33 % of the Gram positive, anaerobic bacteria. The content in agriculturally used soils was reduced even up to 56 %. Nevertheless, the pasture soils contained up to 88 % of the actinomycetes content relative to the reference soils. In soils under greenhouse cultivation the content reduced more than in soils of outdoor cultivations, while differences between conventionally and organically managed systems were not found. The actinomycetes biomass correlated highly significantly ($P < 0.01$) with TOC ($R = 0.484$) and TON ($R = 0.538$) contents but only significantly ($P < 0.05$) with water ($R = 0.284$) content. A highly significant ($P < 0.01$) negative correlation was found with the pH value ($R = – 0.641$).

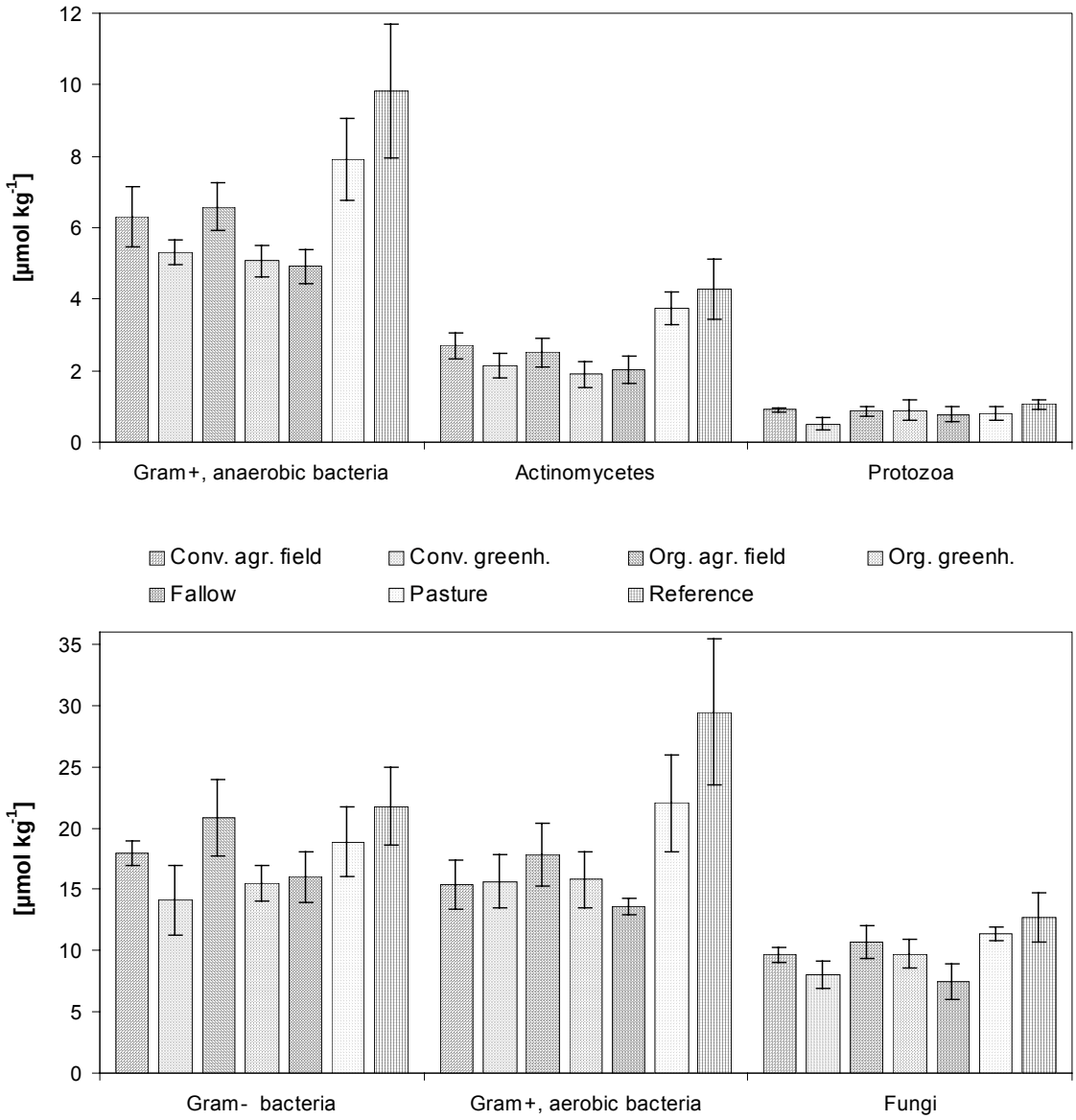

Figure 13: PLFA contents of different microbial taxonomic groups in soils of the land use systems under study with standard error bars; conv.: conventional, org: organic, agr.: agricultural.

The PLFA content indicating protozoa (Figure 13) decreased in the order references > conventional agricultural fields > organic greenhouses > organic agricultural fields > pastures > fallows > conventional greenhouses. However, no significant ($P > 0.05$) differences between the land use systems under study were found in the PLFA content of this taxonomic group. The highest decrease of the protozoa content up to 51 % relative to the reference soils was found in the conventionally managed greenhouse soils whereas every other agriculturally used soil showed a lower content up to 17 %. The pasture soils exhibited the highest content of 77 % and those of the fallows of 73 %. Besides the conventionally managed greenhouse

soils no differences between both the indoor and outdoor and the conventionally and organically managed systems were found. The protozoa biomass correlated significantly ($P < 0.05$) with the soil water content ($R = 0.247$) and showed a highly significant ($P < 0.01$) negative correlation with the pH value ($R = – 0.379$).

The PLFA content indicating Gram negative bacteria (Figure 13) decreased in the order references > organic agricultural fields > pastures > conventional agricultural fields > fallows > organic greenhouses > conventional greenhouses. However, no significant ($P > 0.05$) differences between the investigated land use systems could be found in the PLFA content of this microbial group. The soils of organically managed agricultural fields showed an almost equal content (96 %) of Gram negative bacteria relative to the reference soils followed by the pasture soils (87 %) and those of the conventionally managed agricultural fields (82 %). The highest reduction of up to 35 % exhibited the fallow soils as well as the conventionally and organically managed greenhouse soils. Therefore, the Gram negative bacteria exhibited a tendentially lower content in soils of the greenhouse compared to the outdoor cultivations, while soils of the organically managed systems showed a tendentially higher content than the conventionally managed ones. The biomass of the Gram negative bacteria correlated significantly ($P < 0.05$) with TOC ($R = 0.242$) and TON ($R = 0.293$) contents but no correlation was found with the pH value.

The PLFA content indicating Gram positive, aerobic bacteria (Figure 13) decreased in the order references > pastures > organic agricultural fields > organic greenhouses > conventional greenhouses > conventional agricultural fields > fallows. The reference soils exhibited a significantly ($P < 0.05$) higher PLFA content of Gram positive, aerobic bacteria than those of the other land use systems besides the pastures, which showed a significantly ($P < 0.05$) higher content than the fallow soils. The pasture and fallow soils had the lowest (25 %) and highest (54 %) decrease, respectively, relative to the reference soils. No significant differences with respect to Gram positive, aerobic bacteria could be observed among all agricultural land use systems. The biomass of Gram positive, aerobic bacteria correlated highly significantly ($P < 0.01$) with TOC ($R = 0.678$), TON ($R = 0.611$) and water ($R = 0.359$) contents but showed a highly significant ($P < 0.01$) negative correlation with the pH value ($R = – 0.369$).

The PLFA content indicating fungi (Figure 13) decreased in the order references > pastures > organic agricultural fields > organic greenhouses > conventional agricultural fields

> conventional greenhouses > fallows. However, no significant ($P > 0.05$) differences were found in the PLFA content of this taxonomic group. The fungi content exhibited the lowest decrease in the pasture soils (10 %) and the highest one in the fallow soils (41 %) relative to those of the reference. Indoor cultivations showed a higher decrease in fungi content than outdoor cultivations and the organically managed soils had a slightly higher content than the conventionally managed ones. The fungal biomass correlated highly significantly ($P < 0.01$) with the TON content ($R = 0.308$) and significantly ($P < 0.05$) with the TOC content ($R = 0.289$) but no correlation was found with the pH value.

Summarising, the absolute contents of individual microbial taxonomic groups basically behaved like the microbial biomass resulting from the total PLFA content. The soils of the reference exhibited the highest biomass of all taxonomic groups, followed by those of the pastures. A higher biomass content was observed in soils of agricultural fields compared to greenhouse soils, which might be caused by various climatic factors, different irrigation or tillage systems among indoor and outdoor cultivations. With the exception of slightly higher contents of fungi and Gram negative bacteria no differences were detectable between soils of conventional and organic management systems. Ibekwe and Kennedy (1999) found mono-unsaturated fatty acids in highest amounts in both field and greenhouse samples followed by saturated and branched chain fatty acids. The fallow soils mostly showed the lowest contents of the microbial groups that could be ascribed to changed soil conditions by reasons of land use abandonment that microbes have to cope with. Unfortunately, due to lack of literature no comparison of absolute contents with other investigations about PLFA in soils of different agricultural land use systems was possible. The significant positive correlations of microbial groups with soil organic matter (TOC and TON) are in good agreement with the results of Allison *et al*. (2005) and Yao *et al*. (2000) indicating that sustainable soil organic matter management would also positively influence soil microbial biomass and thus nutrient cycling. Negative correlations of bacteria and protozoa with the pH values are in contrast to the investigations of Allison *et al*. (2005) and Treonis *et al*. (2004), while also no correlation between fungi and pH values was detected by Bååth and Anderson *et al*. (2003) and Treonis *et al*. (2004). However, it is generally known that neutral pH values favour bacterial growth, while fungi tend to dominate at acidic conditions in contrast to other soil microorganisms (Glaser *et al*., 2004).

3.1.4.4 Microbial community composition

In this investigation soils derived from younger quaternary eolian sediments with similar silty texture and developed under the same subtropical climate (Table 3) were selected so that it can be assumed that differences in the microbial community structure were due to the actual land use. The relative contribution of the individual taxonomic groups to the total PLFA content decreased in the order Gram positive, aerobic bacteria ~ Gram negative bacteria > fungi > Gram positive, anaerobic bacteria > actinomycetes > protozoa in soils of all land use systems (Figure 14). Gram negative bacteria exhibited a significantly ($P < 0.05$) higher relative contribution in soils of the fallows, conventionally and organically managed agricultural fields in comparison to those of the conventionally managed greenhouses, pastures and the reference, while compared to the organically cultivated greenhouse soils their contribution was only tendentially but not significantly ($P > 0.05$) higher. Hence, the rapidly growing Gram negative bacteria, which make use of a variety of different readily available carbon sources (Burke *et al*., 2003) caused a conspicuous change in microbial community composition. Light variabilities in the relative contributions within all other microbial groups among the land use systems were not significant ($P > 0.05$). Bossio *et al*. (2006) explained that a low relative abundance of mono-unsaturated fatty acids representing Gram negative bacteria indicates restricted aeration and / or lower carbon availability. The authors found that the differences between wetland and agricultural sites stood for 20 % of all variability in PLFA profiles and was greater than seasonal and spatial variability. Despite a high total organic carbon content in the pasture and reference soils (Table 3, page 37) the relative contribution of Gram negative bacteria was very low (Figure 14). Hence, their higher contribution in soils of the fallows, conventionally and organically managed agricultural fields could not be ascribed to high carbon availability because in these soils the organic carbon content was lower than in soils of the other land use systems (Table 3, page 37). Thus, this fact might be caused by better aeration due to higher tillage activity and resulting lower soil density on the one hand, or due to other climatic conditions on the other hand in soils of the agricultural fields and the fallows, which formerly have been treated as conventionally managed agricultural fields, in comparison to the anthropogenically unaffected, pasture and greenhouse soils. Contradictory, Yao *et al*. (2000) described a switch from a Gram positive dominated population to more Gram negative species being indicative of progressive change

from oligotrophic to more eutrophic conditions. This would mean that agricultural fields were fertilised more when compared to greenhouse soils and consequently, pastures and the reference are tendentially the richest in nutrients.

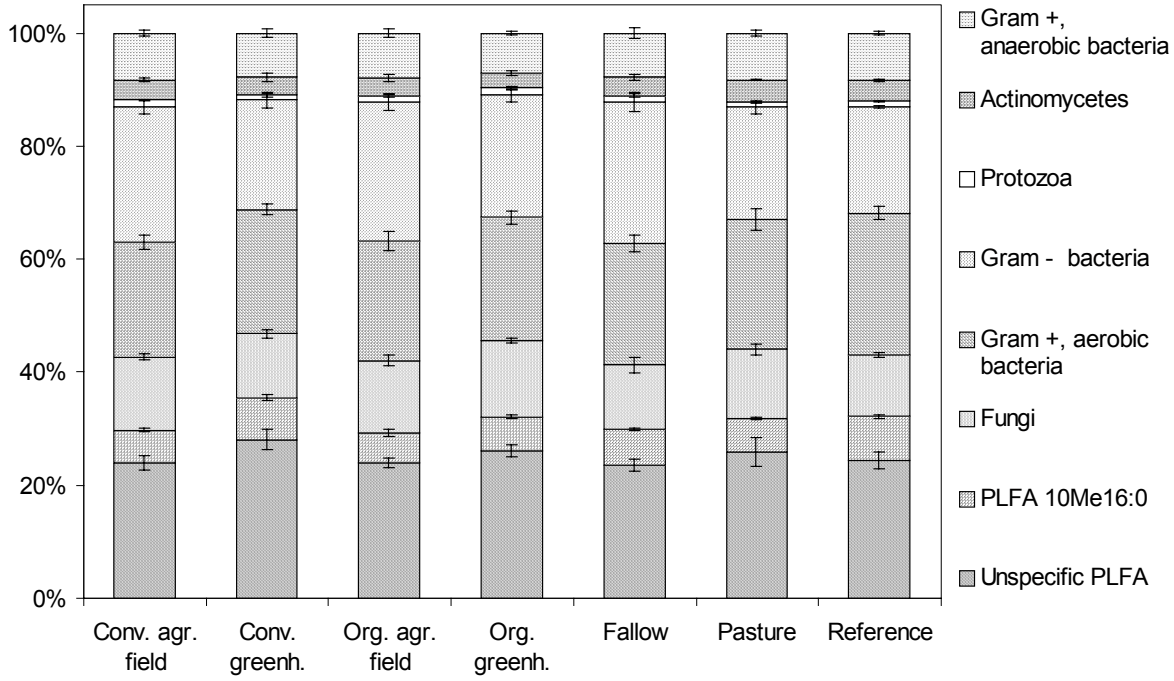

Figure 14: Relative contribution of the individual microbial taxonomic groups in soils of the land use systems under study with standard error bars; conv.: conventional, org: organic, agr.: agricultural, greenh.: greenhouse.

Relative to the reference soils the contribution of Gram positive, anaerobic bacteria at the sum content of all detected microbial taxonomic groups slightly decreased in soils of all land use systems, while that of the actinomycetes decreased more besides pasture soils, in which a light increase was observable (Table 13). With the exception of little decreases in soils of pastures and conventionally cultivated greenhouses the relative contribution of protozoa mainly increased in the remaining land use systems. Gram positive, anaerobic bacteria exhibited the most distinct relative decrease of all land use systems when compared to the reference soils, while the only microbial groups, which showed a clear relative increase were fungi and Gram negative bacteria, although these taxonomic groups absolutely decreased.

Table 13: Percentage changes of relative abundances of the microbial taxonomic groups in soils of the land use systems under study relative to the reference soils with standard errors.

Land use systems	Gram +, anaerobic b.	Actino-mycetes	Protozoa	Gram - bacteria	Gram +, aerobic b.	Fungi
Conv. agr. field	99.1 ± 7.4	97.5 ± 7.3	129.5 ± 6.2	127.1 ± 6.4	80.7 ± 5.0	118.0 ± 4.7
Conv. greenh.	92.7 ± 9.3	89.1 ± 20.2	91.2 ± 34.9	102.5 ± 7.4	87.7 ± 3.0	103.2 ± 6.9
Org. agr. field	94.9 ± 8.6	85.2 ± 15.1	119.0 ± 23.6	130.5 ± 8.1	84.2 ± 7.0	116.8 ± 9.1
Org. greenh.	84.4 ± 2.8	72.4 ± 11.4	134.3 ± 40.3	114.8 ± 6.1	86.7 ± 4.5	123.5 ± 4.4
Fallow	93.4 ± 10.4	87.9 ± 16.3	126.3 ± 33.7	132.1 ± 8.9	86.1 ± 5.9	104.1 ± 13.0
Pasture	99.2 ± 5.5	109.0 ± 3.9	91.4 ± 16.3	105.2 ± 5.7	91.6 ± 7.8	111.9 ± 8.9
Reference	100.0 ± 3.6	100.0 ± 4.8	100.0 ± 12.4	100.0 ± 1.0	100.0 ± 4.7	100.0 ± 3.3

Conv.: conventional, org.: organic, agr.: agricultural, greenh.: greenhouse, b.: bacteria.

Bossio *et al*. (1998) reported that changes over time in the microbial community of two Californian Entisols were of greater magnitude than changes associated with management regimes. In contrast to the present investigation, Bossio *et al*. (1998) were able to discriminate microbial communities with the aid of PLFA profiles in different soils and differentiated those that had developed under various agricultural management systems. Similar results were found in a study about PLFA in tropical soils where the microbial community structure varied measurably on different soil and land use types whereas soil types had greater effects on the community than did the management of these soils (Burke *et al*., 2003). Furthermore, it was demonstrated that soil management had an influence on soil microbial community structure by comparing PLFA profiles in tropical forest and converted Mollisols under pineapple plantations in Tahiti (Waldrop *et al*., 2000). The authors showed that especially biomarkers for Gram positive bacteria were relatively less abundant in plantation than in forest soils. However, this is in contrast to the results of Burke *et al*. (2003) who reported that biomarkers for Gram positive bacteria tended to be relatively more abundant in agricultural treatments. Both Waldrop *et al*. (2000) and Burke *et al*. (2003) agreed that actinomycetes and fungi were more important in agricultural or plantation soils compared to the forest ones. Equal results were obtained for protozoa (Burke *et al*., 2003), while Gram negative bacteria showed no discernable pattern in response to managements (Waldrop *et al*., 2000). Bossio *et al*. (2005) found PLFA profiles, which were primarily sensitive to land use conversion in a landscape in western Kenya but also differentiated soil types and soil management effects on microbial communities. Forest soils were indicative of a higher relative abundance of Gram

negative bacteria and fungi and conversely, agricultural soils indicated higher levels of actinomycetes and Gram positive bacteria (Bossio *et al*., 2005). Ibekwe and Kennedy (1998) found higher relative abundances of branched fatty acids representing Gram positive bacteria in soils of agricultural fields in comparison to greenhouse soils, while fatty acids reported to be of origin of Gram positive bacteria were present in high proportion in both non-rhizosphere and rhizosphere in agricultural soils (Ibekwe and Kennedy 1999). Steenwerth *et al*. (2002) determined higher incidences of markers for Gram positive bacteria, fungi and eukaryotes particularly in perennial grassland sites – which could be comparable with fallows – when compared to agricultural fields. Van der Wal *et al*. (2006) reported that fungal biomass was low at the start of land abandonment and increased during the first two years afterwards. After this initial increase of fungal biomass no further increase was apparent and thus, the authors concluded that this was caused by stopping agricultural management activities (van der Wal *et al*., 2006).

In the present study, it was surprising that large inputs of organic as well as inorganic fertilisers over many years did not cause more pronounced changes in microbial community composition. Also neither the application of pesticides nor the unintended entry of heavy metals through sewage sludge or heavy metal containing mineral fertilisers in conventional cultivations hardly had effects, what possibly could be explained by low concentrations in the soils under study. Furthermore, soil compaction and manure input on pastures did not seem to have any significant effects on these soils. The results may indicate that eventually plant cover, varying climatic conditions due to different irrigation or tillage activities in indoor compared to outdoor cultivations – as described for Gram negative bacteria – had stronger effects in agricultural soils around Buenos Aires. For future studies more soils of different types and texture as well as of other environmental or land use factors likely to influence soil microbial communities are needed to be analysed in order to examine other potential sources responsible for changes in community composition and to check for the applicability of PLFA pattern as indicators of microbial soil biodiversity. In this investigation PLFA analysis generally was capable to detect potential changes in soil microbial community composition. The intensive pressure on land use led to a drastic reduction of the microbial biomass but fortunately the structure of the soil microbial decomposer community kept within a limit.

3.1.4.5 Discriminant and hierarchical cluster analysis

With the aid of a discriminant analysis calculated by the factor scores resulting from the principal component analysis of the determined PLFA, it was not possible to discriminate between soils of the diverse land use systems (data not shown). Hence, it can be concluded that soil microbial community function parameters were more capable to distinguish among soils of the different land use systems than soil microbial community structure ones. These results are in contrast to those of Puglisi *et al*. (2005) who found strong effects after changing environmental conditions of soils by evaluating PLFA patterns using multivariate statistical analyses. Similar to the statistical analysis of the functional parameters (Figure 8, page 56) a hierarchical cluster analysis was performed using the factor scores as variables and the land use systems as label cases with the objective to identify similarities between soils of the land use systems. This cluster analysis (Figure 15) showed strong similarities in PLFA pattern of greenhouse soils, which were very different to those of agricultural fields. Pasture and fallow soils showed almost the same PLFA pattern, while being similar to those of conventional and organic agricultural fields. Reference soils formed a single cluster. Hence, the pasture soils behaved totally different in comparison to the hierarchical cluster analysis of the functional parameters, in which they formed an own cluster. However, good agreement resulted from cultivated soils. The microbial parameters showed similarities between soils of greenhouses, which distinctly differed from those of agricultural fields indicating that not the soils of the same management systems exhibited the highest similarities but those of indoor or outdoor cultivations. In both hierarchical cluster analyses the reference soils were allocated to single clusters showing the most varieties in comparison to anthropogenically affected soils.

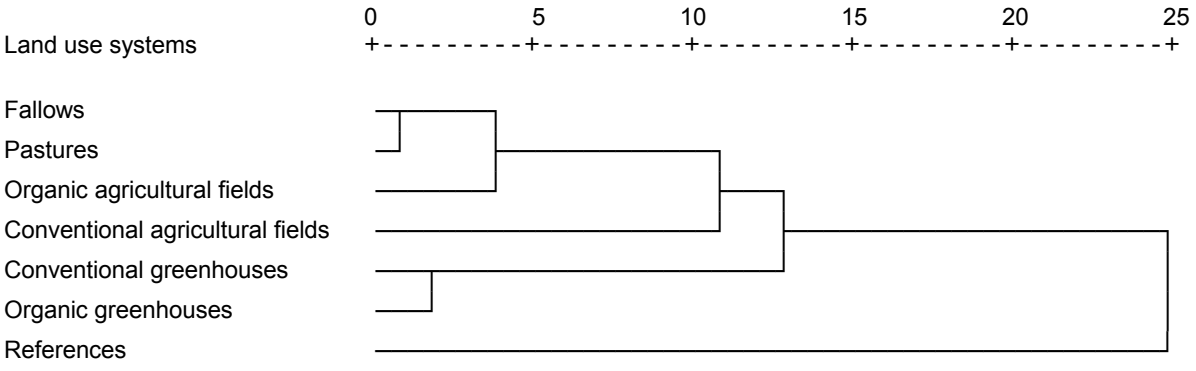

Figure 15: Dendrogram of the hierarchical cluster analysis of the land use systems under study.

3.1.4 Linking soil microbial community structure to function

Schloter *et al*. (2003b) stated that microbial community structure as its own is of no value as soil quality indicator and has to be combined with functional parameters. Another possibility to use structural microbial diversity as an indicator would be to monitor taxonomic groups of microbes, which are known to influence ecosystemary processes (Schloter *et al*., 2003b). Correlation coefficients and significance levels between soil microbial community structure and function parameters of the soils under study are given in Table 14. In contrast to Waldrop *et al*. (2000) who correlated the relative abundances of individual PLFA with enzyme activities, in this study absolute microbial taxonomic group contents were correlated with enzyme activities, respiratory and nitrogen parameters. Because of a low contribution to the microbial community composition the protozoa biomass exhibited the lowest connection to the functional parameters compared to the other microbial groups. Only significantly ($P < 0.05$) negative correlations could be found with substrate-induced respiration and net nitrification. This became more distinct regarding correlations with soil microbial biomass. Those microbial groups showing the lowest contribution to total PLFA content (protozoa and actinomycetes; Figure 14) were not correlated, while those showing a higher contribution were correlated. Hence, Gram positive bacteria representing the highest proportion of the microbial biomass exhibited the most correlations to the soil microbial functional parameters indicating the highest ecological significance in the soils under study. Besides urease activity, metabolic quotient and potential denitrification the biomass of Gram positive, aerobic bacteria showed mainly highly significant ($P < 0.01$) correlations with all analysed functional parameters. Both Gram positive, anaerobic bacteria and actinomycetes exhibited mainly highly significant ($P < 0.01$) positive correlations to acid phosphatase, arylsulfatase, dehydrogenase activity, basal respiration and metabolic quotient and highly significant ($P < 0.01$) negative correlations to potential denitrification. Furthermore, Gram positive, anaerobic bacteria correlated significantly ($P < 0.05$) with microbial biomass and net nitrification. Thus, the taxonomic group of Gram positive bacteria played an important role for nutrient release in the studied soils. Gram positive bacteria are considered being stress tolerators (Waldrop *et al*., 2000), which grow slowly and are able to metabolise complex organic substrates such as lignin and humic acids more readily than Gram negative bacteria (Burke *et al*., 2003). Basal respiration can serve as an indicator of total carbon turn-over and

reflects the availability of soil organic matter (Yao *et al*., 2000). Therefore, due to the higher organic matter content particularly in pasture and reference soils (Table 3, page 37) all Gram positive bacteria and fungi had highly significant ($P < 0.01$) correlations to basal respiration and thus demonstrated good carbon turn-over and availability. This especially applied to Gram positive, anaerobic bacteria and actinomycetes since they were additionally correlated with the metabolic quotient, which is a measure for degradation rates of organic carbon (Anderson, 2003). By reason of the fact that a lot of enzymes are necessary to decompose soil organic matter these groups likewise were often correlated with enzyme activities except for urease. Gram negative bacteria had highly significant ($P < 0.01$) correlations to arylsulfatase and urease activity, microbial biomass and significant ($P < 0.05$) correlations to potential denitrification. This explained why this group is the only one correlating positively with the latter anaerobic process and thus being involved therein in contrast to the even negatively correlated Gram positive bacteria. This corresponded to the general knowledge that mainly Gram negative bacteria e. g. *Paracoccus denitrificans* and pseudomonads e. g. *Pseudomonas stutzeri* besides some Gram positive *Bacillus ssp*. are responsible for denitrification (Suharti and de Vries, 2005). Because of very low ammonium contents (< 0.04 mg kg^{-1}, data not shown) in all soils under study nitrifying Gram negative bacteria like *Nitrosomonas ssp*. or *Nitrobacter ssp*. did not correlate with net nitrogen mineralisation or net nitrification but with urease activity, which delivers ammonium necessary for nitrification by degrading urea. Correlations between Gram positive bacteria and net nitrogen mineralisation as well as net nitrification were not due to direct connections but they were caused by ample TON contents in soils (Table 3, page 37). The same is also true for fungi, exhibiting correlations to microbial biomass as well as to basal and substrate-induced respiration indicating high metabolising activity.

Table 14: Correlation coefficients between PLFA contents of the microbial taxonomic groups and soil microbial functional parameters (N = 69).

Soil microbial functional parameters	Gram +, anaerobic b.	Actino-mycetes	Protozoa	Gram - bacteria	Gram +, aerobic b.	Fungi
Acid phosphatase	0.604 **	0.688 **	0.210	0.017	0.584 **	0.173
Arylsulfatase	0.456 **	0.407 **	0.138	0.493 **	0.565 **	0.534 **
Cellulase	0.196	0.093	-0.062	-0 034	0.407 **	0.118
Dehydrogenase	0.299 *	0.286 *	-0.015	0.066	0.293 *	0.175
Urease	0.190	0.192	0.215	0.366 **	0.220	0.274 *
Basal respiration	0.472 **	0.361 **	-0.108	0.200	0.519 **	0.366 **
Substrate-induced respiration	0.176	-0.094	-0.280 *	0.226	0.425 **	0.306 *
Microbial biomass	0.290 *	0.105	-0.060	0.535 **	0.471 **	0.630 **
Metabolic quotient	0.312 **	0.283 *	-0.064	-0.078	0.211	0.003
Net nitrogen mineralisation	0.160	-0.013	-0.221	0.189	0.275 *	0.156
Net nitrification	0.287 *	0.120	-0.263 *	0.212	0.422 **	0.272 *
Potential denitrification	-0.337 **	-0.646 **	-0.232	0.310 *	-0.141	0.157

* significant ($P < 0.05$), ** highly significant ($P < 0.01$); b.: bacteria.

3.2 Field experiment

3.2.1 Basic soil parameters

The first 10 cm of the soil of the field experiment consisted of 24.5 % clay, 61.1 % silt and 14.4 % sand constituting a silty loam. For this soil depth the initial pH value was 5.9 ± 0.0, total organic carbon (TOC) content 23.2 ± 0.5 g kg^{-1}, total organic nitrogen (TON) content 2.1 ± 0.1 g kg^{-1} and C/N ratio 10.8 ± 0.1.

Figure 16 shows the climate data like temperature and precipitation throughout the field experiment duration. The temperature tendentially increased during the field experiment. With the exception of the sixth sampling day there was no precipitation event before soil sampling. As a result, these samples excepting those of the last sampling day had a similar water content between 14 and 18 %. Before the sixth sampling day a stronger precipitation event took place, by which the samples exhibited a water content between 22 and 24 % besides those of the fallow plots with 18.5 %. Due to the intense temperature increase and marginal precipitation before the last sampling day the samples contained only between 9 and 10 % of water besides the samples of the fallow plots with 12 %.

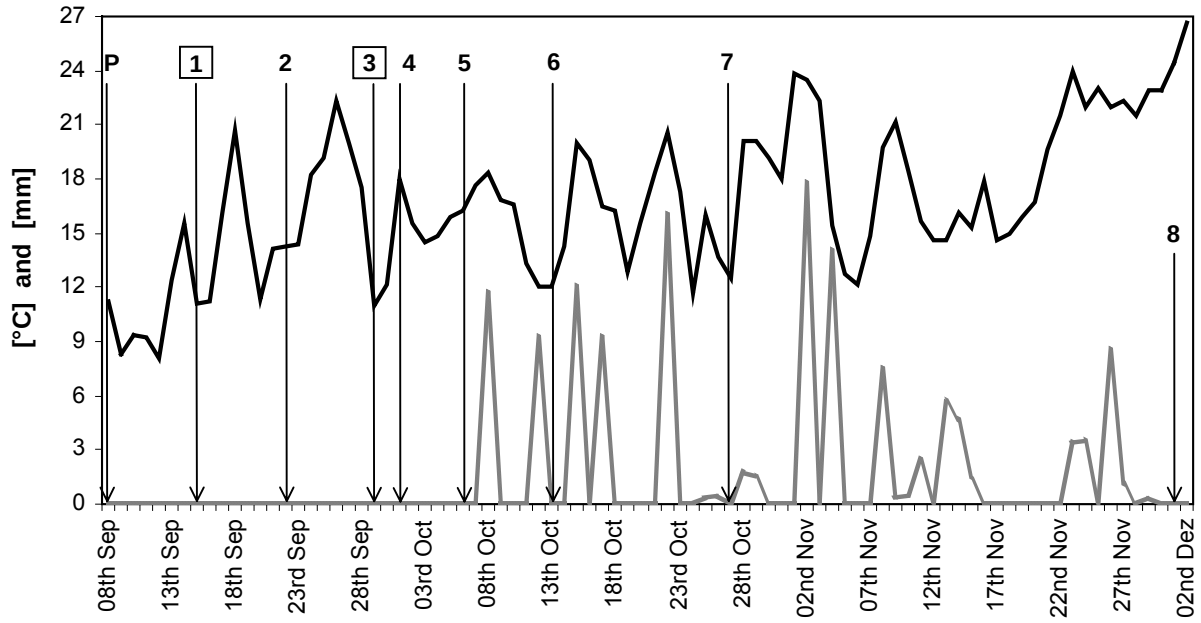

Figure 16: Climate diagram about daily mean temperature (upper black curve) and precipitation (lower grey curve) as well as sampling dates for the duration of the field experiment. Sampling date number 1 was the day of fertiliser and seed application and number 3 that of pesticide application; P: plowing.

The reference plots exhibited tendentially higher TOC and TON contents than the fallow ones (Figure 17) caused by the incorporation of cover plants into the top soil after plowing, which were tendentially higher at the end of the experiment compared to the beginning. Equally, the conventionally managed plots showed similar trends compared to those of the reference ones, while the organic treatments had the mainly significantly ($P < 0.05$) highest contents of TOC and TON due to the humus addition, through which organic carbon and nitrogen was added to the soil. With respect to the C/N ratio, fallow and reference treatments exhibited very similar trends (Figure 17) with values between those of the conventional and organic treatments throughout the field experiment and had tendentially higher values at the end in comparison to the beginning. The significantly ($P < 0.05$) lowest C/N ratio of the conventionally managed plots were attributed to the mineral nitrogen fertilisation, while the mostly significantly ($P < 0.05$) highest values of the organically managed plots were because of the organic fertilisation since the C/N ratio of the humus was higher than that of the soil.

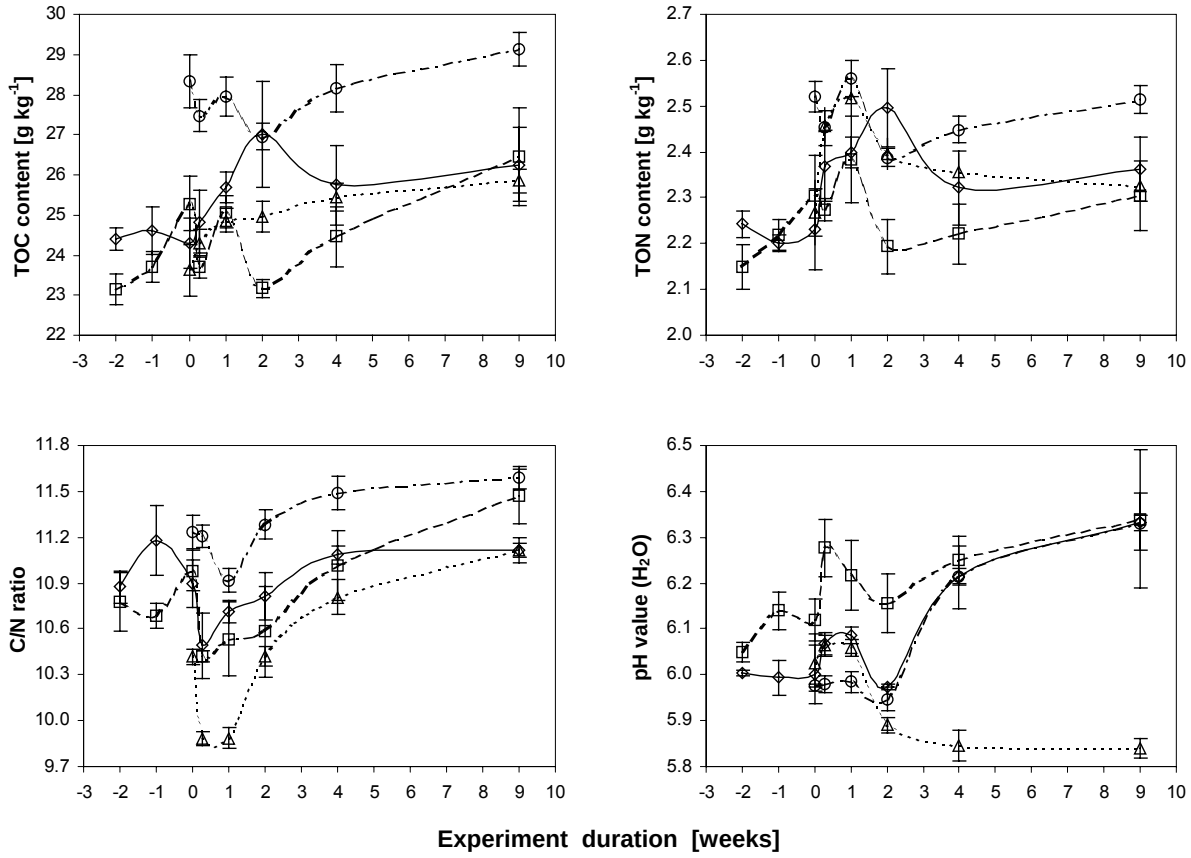

Figure 17: Chronological sequence of the basic soil properties with standard error bars (N = 3).

Until two weeks after pesticide application the fallow plots had the significantly ($P < 0.05$) and thereafter the tendentially highest pH value possibly caused by particulate leaching of alkaline acting cations due to sporadic irrigation of the other treatments, while they showed similar values like the reference and the organically managed plots at the experiment end. At the day of pesticide application as well as two and seven days after, the organic treatments had the significantly ($P < 0.05$) lowest pH values, while thereafter they exhibited a similar trend like the reference plots. At the beginning of the experiment the conventional managed plots showed very similar values like the reference plots, while afterwards they had the significantly ($P < 0.05$) lowest values because through mineral fertilisation phosphate and sulfate were added to the soil and after their solution in soil water the pH values reduced.

3.2.2 Pollutants

The chronological sequences of the active agent contents of endosulfan and metabolite endosulfan sulfate, of chlorothalonil and metabolite 4-hydroxychlorothalonil and of copper imported to the soils by application of THIONEX-L, ISATHALONIL and SUPERCUPROL, respectively, are given in Figure 18. Endosulfan and chlorothalonil exhibited significantly ($P < 0.05$) decreasing contents in soils after application throughout the field experiment, while the contents of their degradation products significantly ($P < 0.05$) increased. The copper content was significantly ($P < 0.05$) higher after application compared to that before, however, during the field experiment the content has not changed significantly ($P > 0.05$).

In the following text the results of the soil microbial functional parameters are discussed with respect to Tables 15 to 20. Tables 15, 17 and 19 show mean values and standard errors of all parameters in every treatment at every sampling date, while Tables 16, 18 and 20 exhibit the same values subtracted by those of the reference plots at the respective sampling date. Therefore, through the subtraction of the reference values climatic factors were eliminated since there was a variation in temperature and precipitation throughout the field experiment (Figure 16, page 78), which also affected the development of the soil microbial functional parameters of the treatments. Various small letters describe significant ($P < 0.05$) differences among diverse treatments on a sampling day (vertical) and capital letters describe significant ($P < 0.05$) temporal differences within the same treatment during the experiment (horizontal).

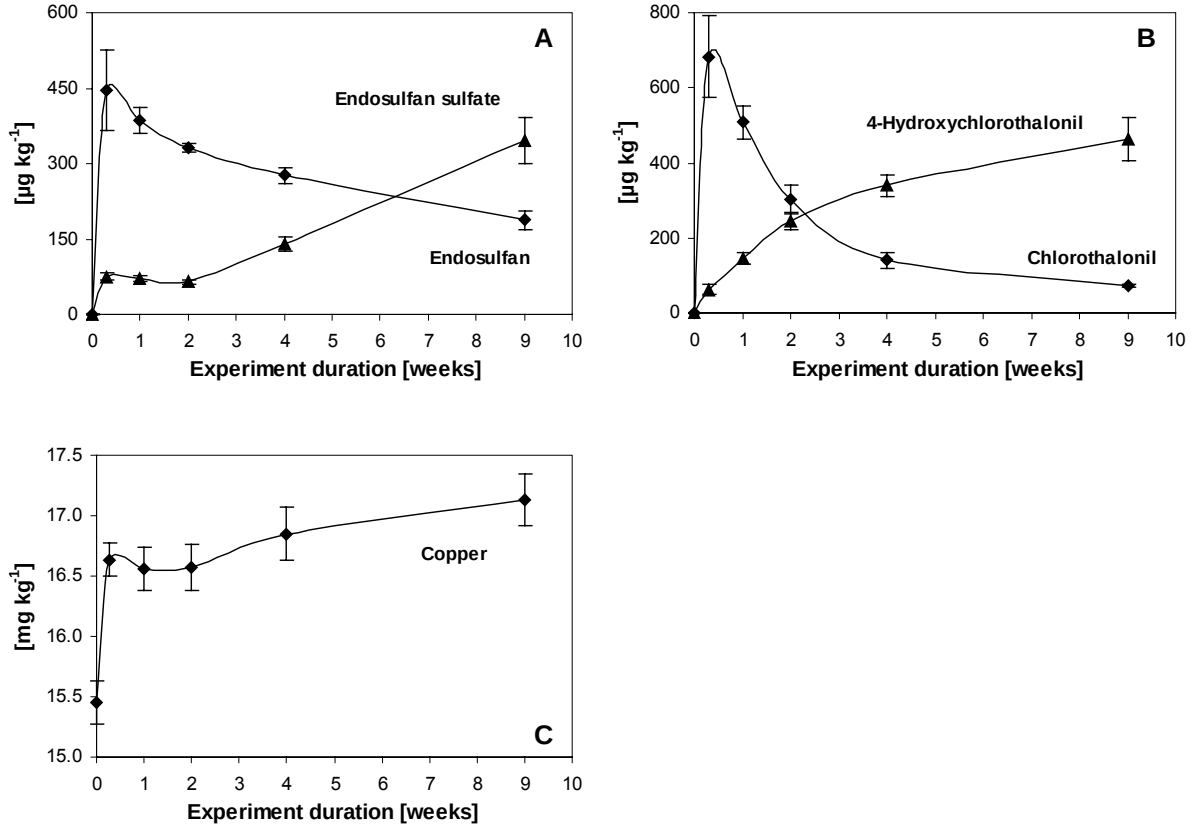

Figure 18: Chronological sequence of the pollutant contents after pesticide application with standard error bars. Figures **A** and **B** exhibit decreasing contents of the active agents endosulfan and chlorothalonil as well as increasing contents of their degradation products endosulfan sulfate and 4-hydroxychlorothalonil of the conventional treatments, respectively, while Figure **C** shows the copper content of the organic treatments (N = 6).

3.2.3 Functional parameters

3.2.3.1 Enzyme activities

The acid phosphatase activity ranged between 0.74 and 1.30 g p-nitrophenol kg^{-1} h^{-1} (Table 15) during the field experiment. The values of the fallow plots showed a similar but mostly lower trend than those of the reference plots. All treatments showed a higher acid phosphatase activity at the end of the experiment than at the beginning. However, by subtraction of the values of the reference plots only the activity of the organic treatments increased and that of the conventional ones decreased. Therefore, the organically managed

plots exhibited a tendentially and at the last sampling day even a significantly ($P > 0.05$) higher acid phosphatase activity than the conventionally managed ones. This could be attributed to the application of mineral phosphate to the conventional treatments, which shifted the enzyme reaction equilibrium to the side of the reaction product phosphate and thus, inhibited the acid phosphatase activity on these treatments. The plots treated with conventional pesticides exhibited the lowest acid phosphatase activity of all conventional treatments two days after pesticide application, and after one week the soils recovered with the exception of the plots treated with both endosulfan and chlorothalonil, which indicated a synergistic inhibiting effect on the acid phosphatase activity. However, no enzyme activity reduction could be detected for the organic plant protection practice.

The arylsulfatase activity ranged between 0.17 and 0.34 g p-nitrophenol kg^{-1} h^{-1} (Table 15) during the field experiment. The arylsulfatase activity in the fallow plots showed a similar increasing development like that in the reference plots. The conventionally managed plots showed a relatively constant arylsulfatase activity within the experiment duration excepting an increase one week after pesticide application but subtracting the reference values the activity decreased. By contrast, in the organic treatments the arylsulfatase activity increased with time and remained constant compared to the reference plots except a strong increase four weeks after pesticide application. Two weeks after pesticide application until the end of the field experiment, a significantly ($P < 0.05$) lower arylsulfatase activity was found in conventionally compared to organically fertilised plots, which was similar but more distinct in comparison to phosphatase activity. This was attributed to the mineral sulfate application on these plots inhibiting the arylsulfatase activity through a shift of the enzyme reaction equilibrium to the side of the reaction product sulfate. Corroborating the trends of the acid phosphatase activity, the conventionally managed plots, which were treated with pesticides, exhibited the lowest arylsulfatase activity of all conventional treatments two days after pesticide application indicating a pesticide-induced inhibition. However, five days later the arylsulfatase activity recovered with the exception of the plots treated with both endosulfan and chlorothalonil indicating a synergistic effect on arylsulfatase activity inhibition. In addition, the plots with copper oxychloride application had the lowest arylsulfatase activity of all organically managed ones except at the experiment end, which indicated an inhibiting effect of the arylsulfatase activity by copper.

Table 15a: Average enzyme activities in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Acid phosphatase activity [g p-NP kg^{-1} h^{-1}]								
F	0.81 ± 0.06 a A	0.82 ± 0.03 a A	1.01 ± 0.09 a A	0.76 ± 0.09 a A	0.87 ± 0.10 a A	0.81 ± 0.07 a A	0.85 ± 0.10 a A	0.94 ± 0.15 a A
R	0.98 ± 0.03 a AB	0.84 ± 0.02 a A	1.00 ± 0.07 a B	0.85 ± 0.03 a AB	0.93 ± 0.04 a AB	0.94 ± 0.09 a AB	0.92 ± 0.05 a AB	1.19 ± 0.02 bcd C
M			0.87 ± 0.00 a AB	0.90 ± 0.03 a AB	0.93 ± 0.04 a ABC	0.86 ± 0.06 a A	0.97 ± 0.03 a BC	1.02 ± 0.02 ab C
M Es			0.87 ± 0.00 a A	0.77 ± 0.03 a A	0.88 ± 0.05 a A	0.86 ± 0.00 a A	0.89 ± 0.07 a A	0.96 ± 0.05 a A
M Cl			0.87 ± 0.00 a B	0.75 ± 0.03 a A	0.93 ± 0.02 a C	0.90 ± 0.01 a BC	0.74 ± 0.01 a A	1.04 ± 0.02 abc D
M Es Cl			0.87 ± 0.00 a BC	0.75 ± 0.02 a A	0.82 ± 0.03 a AB	0.98 ± 0.03 a D	0.91 ± 0.02 a CD	1.07 ± 0.05 abc E
H			0.99 ± 0.03 a B	0.80 ± 0.05 a A	0.83 ± 0.04 a A	1.09 ± 0.03 a BC	1.01 ± 0.08 a B	1.21 ± 0.05 cd C
H Bt			0.99 ± 0.03 a BC	0.75 ± 0.03 a A	1.05 ± 0.04 a C	1.03 ± 0.05 a C	0.87 ± 0.02 a AB	1.30 ± 0.07 d D
H Cu			0.99 ± 0.03 a B	0.78 ± 0.05 a A	1.00 ± 0.05 a B	0.94 ± 0.10 a AB	1.00 ± 0.04 a B	1.20 ± 0.02 cd C
H Bt Cu			0.99 ± 0.03 a A	0.90 ± 0.01 a A	0.93 ± 0.04 a A	0.95 ± 0.03 a A	0.94 ± 0.06 a A	1.27 ± 0.03 d B
Arylsulfatase activity [g p-NP kg^{-1} h^{-1}]								
F	0.21 ± 0.00 a AB	0.20 ± 0.01 a AB	0.22 ± 0.01 a AB	0.22 ± 0.01 cde A	0.23 ± 0.01 bc B	0.20 ± 0.02 abc AB	0.22 ± 0.02 ab AB	0.34 ± 0.02 d C
R	0.24 ± 0.00 b C	0.20 ± 0.00 a A	0.23 ± 0.02 a BC	0.23 ± 0.01 e BC	0.23 ± 0.00 c BC	0.21 ± 0.02 bc AB	0.23 ± 0.01 b BC	0.30 ± 0.02 cd D
M			0.20 ± 0.01 a A	0.23 ± 0.00 de B	0.27 ± 0.01 de C	0.18 ± 0.01 ab A	0.19 ± 0.00 a A	0.23 ± 0.01 ab B
M Es			0.20 ± 0.01 a AB	0.21 ± 0.01 bc AB	0.28 ± 0.01 e C	0.18 ± 0.01 ab A	0.23 ± 0.02 b B	0.23 ± 0.01 a B
M Cl			0.20 ± 0.01 a A	0.18 ± 0.02 a A	0.25 ± 0.02 ce B	0.17 ± 0.01 a A	0.19 ± 0.01 a A	0.21 ± 0.01 a A
M Es Cl			0.20 ± 0.01 a B	0.19 ± 0.00 ab AB	0.19 ± 0.01 a AB	0.18 ± 0.01 ab A	0.20 ± 0.00 ab B	0.23 ± 0.00 ab C
H			0.23 ± 0.01 a B	0.23 ± 0.00 de B	0.21 ± 0.01 ab A	0.22 ± 0.01 c B	0.34 ± 0.00 e D	0.27 ± 0.01 bc C
H Bt			0.23 ± 0.01 a A	0.22 ± 0.01 cde A	0.24 ± 0.01 cd AB	0.22 ± 0.02 c A	0.31 ± 0.02 de C	0.30 ± 0.03 cd BC
H Cu			0.23 ± 0.01 a B	0.21 ± 0.01 cde AB	0.20 ± 0.00 a A	0.21 ± 0.01 bc AB	0.28 ± 0.01 c C	0.28 ± 0.01 c C
H Bt Cu			0.23 ± 0.01 a C	0.21 ± 0.00 cd B	0.20 ± 0.01 a A	0.21 ± 0.01 bc B	0.28 ± 0.01 cd D	0.30 ± 0.00 cd D
Cellulase activity [mg glucose kg^{-1} h^{-1}]								
F	21.7 ± 1.3 a C	19.8 ± 1.6 a BC	17.3 ± 1.0 b AB	15.1 ± 0.5 a A	16.8 ± 2.0 a AB	19.6 ± 0.6 bc BC	17.9 ± 0.8 a AB	18.4 ± 1.1 cd ABC
R	16.8 ± 2.1 a BC	19.8 ± 0.6 a CD	13.8 ± 0.9 a AB	13.8 ± 1.0 a AB	18.8 ± 1.2 a CD	20.5 ± 0.8 c D	19.3 ± 0.9 a CD	12.7 ± 1.1 a AB
M			13.1 ± 0.7 a A	15.6 ± 0.7 a AB	17.0 ± 0.9 a BC	19.6 ± 1.0 bc CD	17.2 ± 1.3 a BCD	20.3 ± 1.5 d D
M Es			13.1 ± 0.7 a A	17.1 ± 0.3 a B	15.9 ± 1.2 a AB	18.4 ± 1.6 abc B	18.4 ± 0.6 a B	17.9 ± 0.7 bcd B
M Cl			13.1 ± 0.7 a A	17.3 ± 1.4 a BCD	14.8 ± 0.8 a AB	19.0 ± 1.1 abc CD	19.2 ± 1.3 a D	15.5 ± 1.6 abc ABC
M Es Cl			13.1 ± 0.7 a A	15.1 ± 0.2 a AB	17.8 ± 1.8 a BC	19.7 ± 0.4 bc C	18.7 ± 0.8 a C	13.6 ± 0.8 a A
H			13.6 ± 0.8 a A	14.7 ± 2.2 a A	18.3 ± 1.0 a A	16.8 ± 0.9 a A	15.7 ± 1.6 a A	14.3 ± 0.6 ab A
H Bt			13.6 ± 0.8 a A	17.3 ± 2.7 a A	17.0 ± 1.6 a A	17.4 ± 0.2 ab A	18.6 ± 2.4 a A	16.2 ± 0.1 abc A
H Cu			13.6 ± 0.8 a A	14.0 ± 1.0 a A	16.7 ± 1.4 a A	16.6 ± 0.7 a A	17.1 ± 1.8 a A	15.7 ± 2.6 abc A
H Bt Cu			13.6 ± 0.8 a A	15.8 ± 2.3 a A	16.4 ± 0.7 a A	17.2 ± 0.6 ab A	17.4 ± 0.6 a A	12.8 ± 1.5 a A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 15b: Average enzyme activities in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks before pesticide application	1 week before pesticide application	day of application	2 days after pesticide application	1 week after pesticide application	2 weeks after pesticide application	4 weeks after pesticide application	9 weeks after pesticide application
Treatments								
Dehydrogenase activity [mg TPF kg^{-1} h^{-1}]								
F	4.5 ± 0.3 a A	4.8 ± 0.2 a A	5.0 ± 0.4 a AB	6.4 ± 0.7 abcd BC	6.8 ± 0.6 de C	4.8 ± 0.6 a A	5.1 ± 0.7 bc AB	3.9 ± 0.5 a A
R	5.4 ± 0.2 a BC	5.2 ± 0.2 a B	4.5 ± 0.3 a B	7.8 ± 0.3 e E	6.5 ± 0.1 bcde D	6.1 ± 0.1 a CD	6.4 ± 0.5 d D	3.3 ± 0.4 a A
M			4.7 ± 0.2 a A	7.0 ± 0.4 bcde A	5.6 ± 0.5 bc A	6.1 ± 0.4 a A	5.1 ± 0.3 c A	4.8 ± 0.9 a A
M Es			4.7 ± 0.2 a A	7.0 ± 0.3 cde C	7.3 ± 0.5 e C	5.3 ± 0.1 a AB	6.0 ± 0.2 cd B	5.2 ± 0.3 a AB
M Cl			4.7 ± 0.2 a A	6.2 ± 0.5 abcd CD	6.6 ± 0.2 cde D	5.5 ± 0.4 a ABC	6.1 ± 0.2 cd BCD	5.2 ± 0.3 a AB
M Es Cl			4.7 ± 0.2 a B	5.3 ± 0.2 a B	6.2 ± 0.3 bcd C	5.5 ± 0.1 a BC	5.1 ± 0.2 c B	3.5 ± 0.5 a A
H			6.0 ± 0.9 a A	6.6 ± 0.3 bcde A	5.4 ± 0.2 ab A	5.6 ± 0.6 a A	6.6 ± 0.3 d A	4.7 ± 0.2 a A
H Bt			6.0 ± 0.9 a ABC	7.3 ± 0.5 de C	7.4 ± 0.5 e C	5.1 ± 0.5 a AB	6.6 ± 0.6 d BC	4.1 ± 0.9 a A
H Cu			6.0 ± 0.9 a B	5.8 ± 0.1 ab B	4.4 ± 0.1 a A	5.5 ± 0.0 a B	4.0 ± 0.4 ab A	3.9 ± 0.3 a A
H Bt Cu			6.0 ± 0.9 a B	5.9 ± 0.3 abc B	5.7 ± 0.4 bc B	4.9 ± 0.3 a B	3.5 ± 0.2 a A	5.4 ± 0.2 a B
Urease activity [mg NH_4^+ kg^{-1} h^{-1}]								
F	28.5 ± 3.5 a A	29.3 ± 4.1 a A	32.1 ± 4.6 a A	28.4 ± 3.4 a A	26.3 ± 2.5 a A	31.9 ± 3.9 a A	32.6 ± 4.5 a A	33.9 ± 3.4 c A
R	27.5 ± 1.0 a C	23.2 ± 1.9 a A	24.3 ± 0.9 a ABC	26.1 ± 1.1 a ABC	23.8 ± 0.7 a AB	24.8 ± 0.9 a ABC	26.9 ± 1.2 a BC	33.7 ± 1.2 c D
M			23.2 ± 3.0 a A	29.1 ± 2.2 a A	28.1 ± 2.3 a A	25.4 ± 2.6 a A	25.3 ± 2.1 a A	28.0 ± 1.9 ab A
M Es			23.2 ± 3.0 a A	25.0 ± 2.6 a A	21.9 ± 2.3 a A	23.8 ± 1.8 a A	23.8 ± 1.9 a A	25.8 ± 2.8 a A
M Cl			23.2 ± 3.0 a A	27.9 ± 3.0 a A	29.0 ± 0.6 a A	23.8 ± 1.5 a A	24.0 ± 0.4 a A	27.2 ± 1.1 a A
M Es Cl			23.2 ± 3.0 a A	26.1 ± 2.9 a A	22.8 ± 4.6 a A	26.6 ± 2.8 a A	24.4 ± 0.8 a A	28.0 ± 0.7 ab A
H			24.9 ± 0.9 a A	24.8 ± 1.5 a A	22.8 ± 0.6 a A	25.6 ± 2.5 a A	24.6 ± 1.0 a A	33.9 ± 1.1 c B
H Bt			24.9 ± 0.9 a AB	26.0 ± 1.0 a B	25.4 ± 1.3 a B	22.9 ± 0.0 a A	24.3 ± 0.5 a AB	32.8 ± 1.4 bc C
H Cu			24.9 ± 0.9 a A	26.0 ± 3.1 a A	24.2 ± 1.7 a A	23.8 ± 2.8 a A	25.2 ± 0.8 a A	30.6 ± 1.1 abc A
H Bt Cu			24.9 ± 0.9 a BC	23.2 ± 0.7 a AB	26.9 ± 1.1 a CD	21.5 ± 0.4 a A	28.1 ± 0.7 a D	34.8 ± 1.4 c E

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 16a: Average enzyme activities subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Acid phosphatase activity [g p-NP kg^{-1} h^{-1}]								
F	–0.16 ± 0.06 a A	–0.02 ± 0.03 a A	0.01 ± 0.09 a A	–0.08 ± 0.09 a A	–0.06 ± 0.10 a A	–0.14 ± 0.07 a A	–0.07 ± 0.10 a A	–0.25 ± 0.15 a A
R	0.00 ± 0.03 a A	0.00 ± 0.02 a A	0.00 ± 0.07 a A	0.00 ± 0.03 a A	0.00 ± 0.04 a A	0.00 ± 0.09 a A	0.00 ± 0.05 a A	0.00 ± 0.02 bcd A
M			–0.13 ± 0.00 a A	0.05 ± 0.03 a C	0.00 ± 0.04 a BC	–0.08 ± 0.06 a AB	0.05 ± 0.03 a C	–0.17 ± 0.02 ab A
M Es			–0.13 ± 0.00 a A	–0.08 ± 0.03 a A	–0.04 ± 0.05 a A	–0.08 ± 0.00 a A	–0.04 ± 0.07 a A	–0.23 ± 0.05 a A
M Cl			–0.13 ± 0.00 a B	–0.10 ± 0.03 a B	0.00 ± 0.02 a C	–0.04 ± 0.01 a C	–0.19 ± 0.01 a A	–0.15 ± 0.02 abc AB
M Es Cl			–0.13 ± 0.00 a A	–0.10 ± 0.02 a A	–0.11 ± 0.03 a A	0.04 ± 0.03 a B	–0.01 ± 0.02 a B	–0.12 ± 0.05 abc A
H			–0.01 ± 0.03 a AB	–0.05 ± 0.05 a A	–0.10 ± 0.04 a A	0.15 ± 0.03 a C	0.09 ± 0.08 a BC	0.02 ± 0.05 cd ABC
H Bt			–0.01 ± 0.03 a AB	–0.10 ± 0.03 a A	0.12 ± 0.04 a B	0.09 ± 0.05 a B	–0.06 ± 0.02 a A	0.11 ± 0.07 d B
H Cu			–0.01 ± 0.03 a A	–0.07 ± 0.05 a A	0.07 ± 0.05 a A	0.00 ± 0.10 a A	0.08 ± 0.04 a A	0.01 ± 0.02 cd A
H Bt Cu			–0.01 ± 0.03 a A	0.05 ± 0.01 a A	0.01 ± 0.04 a A	0.01 ± 0.03 a A	0.02 ± 0.06 a A	0.08 ± 0.03 d A
Arylsulfatase activity [g p-NP kg^{-1} h^{-1}]								
F	–0.03 ± 0.00 a A	0.00 ± 0.01 a A	–0.01 ± 0.01 a A	–0.01 ± 0.01 cde A	0.00 ± 0.01 bc A	–0.02 ± 0.02 abc A	–0.03 ± 0.02 ab A	0.04 ± 0.02 d B
R	0.00 ± 0.00 b A	0.00 ± 0.00 a A	0.00 ± 0.02 a A	0.00 ± 0.01 e A	0.00 ± 0.00 c A	0.00 ± 0.02 bc A	0.00 ± 0.01 b A	0.00 ± 0.02 cd A
M			–0.03 ± 0.01 a BC	0.00 ± 0.00 de C	0.03 ± 0.01 de D	–0.03 ± 0.01 ab B	–0.04 ± 0.00 a B	–0.08 ± 0.01 ab A
M Es			–0.03 ± 0.01 a B	–0.03 ± 0.01 bc B	0.04 ± 0.01 e C	–0.03 ± 0.01 ab B	–0.01 ± 0.02 b B	–0.08 ± 0.01 a A
M Cl			–0.03 ± 0.01 a B	–0.05 ± 0.02 a B	0.02 ± 0.02 ce C	–0.04 ± 0.01 a B	–0.04 ± 0.01 a B	–0.09 ± 0.01 a A
M Es Cl			–0.03 ± 0.01 a B	–0.05 ± 0.00 ab B	–0.04 ± 0.01 a B	–0.03 ± 0.01 ab B	–0.03 ± 0.00 ab B	–0.07 ± 0.00 ab A
H			0.00 ± 0.01 a B	0.00 ± 0.00 de B	–0.03 ± 0.01 ab A	0.01 ± 0.01 c B	0.11 ± 0.00 e C	–0.04 ± 0.01 bc A
H Bt			0.00 ± 0.01 a A	–0.01 ± 0.01 cde A	0.01 ± 0.01 cd A	0.01 ± 0.02 c A	0.08 ± 0.02 de B	0.00 ± 0.03 cd A
H Cu			0.00 ± 0.01 a B	–0.02 ± 0.01 cde AB	–0.04 ± 0.00 a A	0.00 ± 0.01 bc B	0.04 ± 0.01 c C	–0.02 ± 0.01 c AB
H Bt Cu			0.00 ± 0.01 a B	–0.02 ± 0.00 cd A	–0.04 ± 0.01 a A	0.00 ± 0.01 bc B	0.05 ± 0.01 cd C	0.00 ± 0.00 cd B
Cellulase activity [mg glucose kg^{-1} h^{-1}]								
F	4.9 ± 1.3 a CD	0.0 ± 1.6 a AB	3.5 ± 1.0 b BCD	1.3 ± 0.5 a ABC	–2.0 ± 2.0 a A	–0.9 ± 0.6 bc A	–1.4 ± 0.8 a A	5.7 ± 1.1 cd D
R	0.0 ± 2.1 a A	0.0 ± 0.6 a A	0.0 ± 0.9 a A	0.0 ± 1.0 a A	0.0 ± 1.2 a A	0.0 ± 0.8 c A	0.0 ± 0.9 a A	0.0 ± 1.1 a A
M			–0.8 ± 0.7 a AB	1.8 ± 0.7 a B	–1.8 ± 0.9 a A	–0.9 ± 1.0 bc AB	–2.1 ± 1.3 a A	7.6 ± 1.5 d C
M Es			–0.8 ± 0.7 a A	3.3 ± 0.3 a B	–2.8 ± 1.2 a A	–2.1 ± 1.6 abc A	–0.9 ± 0.6 a A	5.2 ± 0.7 bcd B
M Cl			–0.8 ± 0.7 a ABC	3.5 ± 1.4 a D	–4.0 ± 0.8 a A	–1.5 ± 1.1 abc AB	–0.1 ± 1.3 a BCD	2.9 ± 1.6 abc CD
M Es Cl			–0.8 ± 0.7 a A	1.3 ± 0.2 a A	–1.0 ± 1.8 a A	–0.7 ± 0.4 bc A	–0.6 ± 0.8 a A	0.9 ± 0.8 a A
H			–0.3 ± 0.8 a A	1.0 ± 2.2 a A	–0.4 ± 1.0 a A	–3.6 ± 0.9 a A	–3.6 ± 1.6 a A	1.6 ± 0.6 ab A
H Bt			–0.3 ± 0.8 a A	3.5 ± 2.7 a A	–1.8 ± 1.6 a A	–3.0 ± 0.2 ab A	–0.8 ± 2.4 a A	3.5 ± 0.1 abc A
H Cu			–0.3 ± 0.8 a A	0.2 ± 1.0 a A	–2.0 ± 1.4 a A	–3.8 ± 0.7 a A	–2.2 ± 1.8 a A	3.0 ± 2.6 abc A
H Bt Cu			–0.3 ± 0.8 a A	2.0 ± 2.3 a A	–2.4 ± 0.7 a A	–3.3 ± 0.6 ab A	–2.0 ± 0.6 a A	0.1 ± 1.5 a A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 16b: Average enzyme activities subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Dehydrogenase activity [mg TPF kg^{-1} h^{-1}]								
F	–0.9 ± 0.3 a A	–0.4 ± 0.2 a A	0.5 ± 0.4 a A	–1.4 ± 0.7 abcd A	0.4 ± 0.6 de A	–1.3 ± 0.6 a A	–1.3 ± 0.7 bc A	0.6 ± 0.5 a A
R	0.0 ± 0.2 a A	0.0 ± 0.2 a A	0.0 ± 0.3 a A	0.0 ± 0.3 e A	0.0 ± 0.1 bcde A	0.0 ± 0.1 a A	0.0 ± 0.5 d A	0.0 ± 0.4 a A
M			0.2 ± 0.2 a AB	–0.8 ± 0.4 bcde A	–0.9 ± 0.5 bc A	0.0 ± 0.4 a AB	–1.2 ± 0.3 c A	1.5 ± 0.9 a B
M Es			0.2 ± 0.2 a BC	–0.8 ± 0.3 cde A	0.9 ± 0.5 e C	–0.8 ± 0.1 a A	–0.4 ± 0.2 cd AB	1.8 ± 0.3 a D
M Cl			0.2 ± 0.2 a B	–1.6 ± 0.5 abcd A	0.2 ± 0.2 cde B	–0.6 ± 0.4 a AB	–0.3 ± 0.2 cd B	1.8 ± 0.3 a C
M Es Cl			0.2 ± 0.2 a C	–2.4 ± 0.2 a A	–0.2 ± 0.3 bcd C	–0.7 ± 0.1 a BC	–1.2 ± 0.2 c B	0.2 ± 0.5 a C
H			1.4 ± 0.9 a B	–1.1 ± 0.3 bcde A	–1.0 ± 0.2 ab A	–0.5 ± 0.6 a A	0.2 ± 0.3 d AB	1.4 ± 0.2 a B
H Bt			1.4 ± 0.9 a A	–0.5 ± 0.5 de A	0.9 ± 0.5 e A	–1.0 ± 0.5 a A	0.2 ± 0.6 d A	0.8 ± 0.9 a A
H Cu			1.4 ± 0.9 a C	–1.9 ± 0.1 ab A	–2.1 ± 0.1 a A	–0.6 ± 0.0 a B	–2.4 ± 0.4 ab A	0.5 ± 0.3 a BC
H Bt Cu			1.4 ± 0.9 a C	–1.9 ± 0.3 abc AB	–0.8 ± 0.4 bc B	–1.2 ± 0.3 a B	–2.9 ± 0.2 a A	2.1 ± 0.2 a C
Urease activity [mg NH_4^+ kg^{-1} h^{-1}]								
F	0.9 ± 3.5 a A	6.1 ± 4.1 a A	7.9 ± 4.6 a A	2.3 ± 3.4 a A	2.5 ± 2.5 a A	7.1 ± 3.9 a A	5.6 ± 4.5 a A	0.2 ± 3.4 c A
R	0.0 ± 1.0 a A	0.0 ± 1.9 a A	0.0 ± 0.9 a A	0.0 ± 1.1 a A	0.0 ± 0.7 a A	0.0 ± 0.9 a A	0.0 ± 1.2 a A	0.0 ± 1.2 c A
M			–1.1 ± 3.0 a A	3.0 ± 2.2 a A	4.3 ± 2.3 a A	0.6 ± 2.6 a A	–1.7 ± 2.1 a A	–5.6 ± 1.9 ab A
M Es			–1.1 ± 3.0 a A	–1.1 ± 2.6 a A	–1.9 ± 2.3 a A	–1.0 ± 1.8 a A	–3.1 ± 1.9 a A	–7.8 ± 2.8 a A
M Cl			–1.1 ± 3.0 a AB	1.8 ± 3.0 a BC	5.2 ± 0.6 a C	–1.0 ± 1.5 a AB	–3.0 ± 0.4 a AB	–6.5 ± 1.1 a A
M Es Cl			–1.1 ± 3.0 a A	0.0 ± 2.9 a A	–1.1 ± 4.6 a A	1.8 ± 2.8 a A	–2.5 ± 0.8 a A	–5.7 ± 0.7 ab A
H			0.6 ± 0.9 a A	–1.3 ± 1.5 a A	–1.0 ± 0.6 a A	0.8 ± 2.5 a A	–2.3 ± 1.0 a A	0.2 ± 1.1 c A
H Bt			0.6 ± 0.9 a A	–0.2 ± 1.0 a A	1.6 ± 1.3 a A	–1.9 ± 0.0 a A	–2.6 ± 0.5 a A	–0.9 ± 1.4 bc A
H Cu			0.6 ± 0.9 a A	–0.1 ± 3.1 a A	0.4 ± 1.7 a A	–1.0 ± 2.8 a A	–1.8 ± 0.8 a A	–3.1 ± 1.1 abc A
H Bt Cu			0.6 ± 0.9 a B	–2.9 ± 0.7 a A	3.1 ± 1.1 a B	–3.3 ± 0.4 a A	1.2 ± 0.7 a B	–1.1 ± 1.4 c B

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

The cellulase activity ranged between 12.7 and 21.7 mg glucose kg^{-1} h^{-1} (Table 15) during the field experiment. The fallow and reference plots exhibited similar trends with the exception of the day of pesticide application and nine weeks after when the activity of the fallow plots was significantly ($P < 0.05$) higher than in the reference ones. Because of high fluctuation and standard errors of the cellulase activity it was not possible to detect certain trends considering time and different management systems. However, the cellulase activity was tendentially higher in all treatments at the end of the experiment compared to the beginning both with original values and those subtracting the reference values. Moreover, the organically managed plots had a tendentially lower cellulase activity two and four weeks after pesticide application compared to the conventionally managed ones. On the one hand, this result was surprising since the cellulase activity was expected to increase caused by the higher supply of organic carbon, while on the other hand, this could be attributed to fewer readily available nutrients after humus addition in contrast to mineral fertilisation wherefore microbes produced less enzymes. At the end of the field experiment the cellulase activity of the conventionally managed plots decreased in the order plots treated with mineral fertiliser > mineral fertiliser plus endosulfan > mineral fertiliser plus chlorothalonil > mineral fertiliser plus both pesticides. This again indicated a synergistic inhibiting effect on cellulase activity by the conventionally used pesticides. However, no effects could be detected for the organic plant protection practice.

The dehydrogenase activity ranged between 3.3 and 7.8 mg triphenylformazane kg^{-1} h^{-1} (Table 15) during the field experiment. The activity of the fallow and reference plots showed a similar development but the variation of the fallow was not as high as that of the reference. Temporal fluctuation of the dehydrogenase activity within one treatment was higher than differences between the diverse treatments. After a strong increase two days after pesticide application the dehydrogenase activity of the conventional treatments almost returned to the initial values at the experiment end, while the values subtracting those of the reference plots were higher at the end of the field experiment compared to the beginning. The organically managed plots had a lower dehydrogenase activity at the end compared to the beginning of the experiment whereas the plots treated with copper oxychloride exhibited a tendentially lower activity than the remaining ones being significant ($P < 0.05$) four weeks after pesticide application. After strong fluctuations and subtracting the values of the reference plots the

values of the organic treatments mainly slightly decreased compared to the initial ones. The plots treated with endosulfan and chlorothalonil exhibited the tendentially lowest values of the conventional treatments throughout the experiment, while suppressed dehydrogenase activity due to copper application could be observed for the organic plant protection.

The urease activity ranged between 21.5 and 34.8 mg ammonium kg^{-1} h^{-1} (Table 15) during the field experiment. The fallow plots exhibited tendentially the highest urease activity caused by the highest pH values (Figure 17, page 79) since urease activity has its optimum in alkaline medium (Klose and Tabatabai, 1999). Towards the experiment end, the pH value of the reference and organic treatments returned to the initial state and thus, the urease activity approximated to those of the fallow plots. Both conventional and organic treatments showed increasing developments and had higher values at the end of the experiment compared to the beginning, which was even significant ($P < 0.05$) for the latter ones. Subtracting the values of the reference plots the urease activity was reduced in the conventional and organic treatments in comparison to the initial ones. At the experiment end, the conventionally managed plots showed a significantly ($P < 0.05$) lower urease activity than the organically managed ones, which was attributed to different fertiliser application. Ammonium of the mineral fertiliser inhibited the urease activity in conventional treatments through a shift of the enzyme reaction equilibrium to the side of the reaction product ammonium. Hence, the activity was higher in the organic than in the conventional plots. Two and seven days after pesticide application the plots treated with endosulfan had the lowest urease activity, while thereafter no differences among the conventional treatments were detectable. Moreover, no effects could be detected for the organic plant protection practice.

Summarising, the reported ranges of individual enzyme activities were in the same order of magnitude like those of the land use system soils and thus, they can be considered typical for the assayed soils of agricultural use. In general, the significantly ($P < 0.05$) lowest enzyme activities were found in soils of conventional treatments due to mineral fertiliser application inhibiting enzyme activity through addition of those substances, which are released by soil enzymatic reactions. By contrast, in organic treatments enzyme activities were higher since the applied humus as an additional organic carbon source had to be mineralised in order to release plant available nutrients. The fallow plots showed intermediate values with strong variations when compared to the other treatments. With regard to pesticide and heavy metal

applications to the soils only tendential effects on soil microbial functions could be detected in contrast to literature where strong impacts of pesticides on soil microbes were reported.

Omar and Abdel-Sater (2001) examined the influence of the herbicide brominal and the insecticide selecron on a clayey botanical garden soil in Assiut, Egypt. They reported an inhibition of the cellulase activity, while the acid phosphatase activity was promoted at field application rates and delayed at higher application doses of the two pesticides. The effect on the arylsulfatase activity fluctuated between promotion and inhibition, but inhibition was predominant (Omar and Abdel-Sater, 2001). Sannino and Gianfreda (2001) investigated the influence of the herbicides glyphosate, paraquat, atrazine and of the insecticide carbaryl on the activities of invertase, urease and acid phosphatase in Italian soils with a wide range of physical and chemical properties. The authors found general activation effects for urease, while inhibitory effects were observed for the acid phosphatase activity in the presence of glyphosate and paraquat and raises with atrazine and carbaryl. Contradictory, the invertase activity was increased by glyphosate and paraquat and decreased by atrazine and carbaryl (Sannino and Gianfreda, 2001). The activities of dehydrogenase and acid phosphatase were suppressed by fungicide applications of captan and benomyl on terrestrial microcosms with silty loam, while the urease activity was increased (Chen *et al*., 2001a). Beulke and Malkomes (2001) found activating as well as inhibiting effects and strongly inhibiting effects in the dehydrogenase activity of two German loamy sand soils treated with the herbicides dinoterb and metazachlor, respectively, while the effects in the soil having the same physico-chemical properties but markedly lower organic carbon content in comparison to the other were much stronger. Soil enzyme activity was reduced significantly in the order arylsulfatase > alkaline phosphatase > urease > xylanase in all particle-size fractions of a Calcaric Phaeozem exposed to four heavy metal pollution loads (control, 300 mg kg^{-1} Zn, 100 mg kg^{-1} Cu, 50 mg kg^{-1} Ni, 50 mg kg^{-1} V and 3 mg kg^{-1} Cd as well as two- and threefold contents) (Kandeler *et al*., 2000).

3.2.3.2 Respiratory parameters

The basal respiration rate ranged between 4.2 and 8.2 mg carbon dioxide (CO_2) kg^{-1} h^{-1} (Table 17) during the field experiment. The values of the fallow plots varied strongly from the beginning to the end of the experiment and were mostly significantly higher than those of the reference plots. After a strong reduction one week after pesticide application the basal

respiration rate of the conventionally and organically managed plots tendentially increased both with and without subtraction of the reference values and showed higher values at the end of the experiment. However, the basal respiration rate of the organically managed plots varied stronger and tended to be higher than of the conventional ones, which was significant ($P < 0.05$) two weeks after pesticide application. Concerning the applications of the pesticides no differences could be detected both in conventional and organic treatments.

The substrate-induced respiration rate ranged between 44.3 and 80.2 mg CO_2 kg^{-1} h^{-1} (Table 17) during the field experiment. The values of the fallow treatments mainly were significantly ($P < 0.05$) the lowest throughout the experiment and showed a similar trend like the reference ones, which had the second lowest values. The developments of the organically and conventionally managed plots were very similar and showed strong fluctuations until two weeks after pesticide application. The substrate-induced respiration rate of both managements were lower at the end of the experiment compared to the beginning, while subtracting that of the reference plots the values nearly returned to the initial state. However, at the beginning of the experiment the organic treatments exhibited significantly ($P < 0.05$) higher values due to the addition of easily degradable organic matter in the form of humus, which changed towards the end of the experiment as the values of the conventional ones tended to be the highest. Concerning pesticide applications no effects could be detected on both management systems.

The microbial biomass carbon content (C_{mic}) ranged between 0.68 and 1.40 g C_{mic} kg^{-1} (Table 17) during the field experiment. The microbial biomass showed a similar trend in the fallow and reference treatments whereas it was lower in the fallow ones. After strong fluctuations the biomass carbon content for both management systems exhibited higher values at the end compared to the beginning of the field experiment, while subtracting those of the reference plots they decreased at the end. Differences between the two management systems were not detectable. Until four weeks after pesticide application the plots only treated with mineral fertiliser exhibited mainly the lowest microbial biomass carbon content when compared to all conventionally managed plots indicating activation effects of the pesticides. At the end of the experiment when the pesticides distinctly were degraded the plots with conventional pesticide application exhibited lower values compared to those only treated with mineral fertiliser, which was significant ($P < 0.05$) for the plots with both

pesticide applications. This result indicated that at least a combined pesticide application reduced soil microbial biomass even after pesticide degradation. Hence, a negative pesticide effect in the longer term can be assumed, especially when pesticides are applied repeatedly with subsequent cultivation periods. Regarding the organically managed plots, those treated with copper oxychloride mostly exhibited the lowest biomass being significant ($P < 0.05$) four weeks after application and indicating inhibiting effects like the conventional treatments.

The metabolic quotient ranged between 3.9 and 9.9 mg CO_2 (g C_{mic})$^{-1}$ h^{-1} (Table 17) during the field experiment. Equal to the basal respiration the values of the fallow plots varied strongly being significantly ($P < 0.05$) the highest and exhibited similar trends as those of the reference plots. With the exception of a very high value of the mineral fertilised plots four weeks after pesticide application all conventional treatments showed the same trend with small fluctuations and similar values as those at the beginning of the experiment, while they increased subtracting the values of the reference plots until the end of the field experiment compared to the beginning. The metabolic quotient of the organic treatments varied stronger during the experiment especially when those of the reference plots were subtracted. The values were similar at the end of the experiment compared to the beginning and subtracting those of the reference plots they were higher. Similar to the basal respiration the organic treatments tendentially exhibited a mainly significantly ($P < 0.05$) higher metabolic quotient than the conventional ones since two weeks after pesticide application. Until two weeks after experiment beginning the plots treated with conventional pesticides had a tendentially lower and four weeks after a significantly ($P < 0.05$) lower metabolic quotient when compared to the minerally fertilised ones, which indicated activating effects by endosulfan as well as by chlorothalonil. With respect to the organically managed plots those treated with copper oxychloride exhibited a tendentially higher metabolic quotient compared to the remaining ones, which indicated an inhibiting effect caused by copper.

Summarising, the reported ranges of the respiratory parameters were in the same order of magnitude like those of the land use system samples and thus, they also can be considered typical at least for pampean Mollisols under agricultural use. The values of the respiratory parameters were tendentially higher in organic treatments compared to the conventional ones because through humus fertilisation easily degradable organic carbon was added to the soil, which could be metabolised rapidly to carbon dioxide. The fallow plots exhibited

strongly fluctuating values in comparison to the other treatments indicating a high natural variability, which might be climatically driven (Figure 16). With respect to pesticide as well as heavy metal applications to the soils again effects on soil microbial functions could be detected hardly in contrast to literature, in which several impacts of pesticides on these parameters were reported. Probably the high natural fertility of Mollisols could compensate for negative effects of pesticides on soil microbial functions, which will be only obvious after advanced soil degradation.

Beulke and Malkomes (2001) determined a lower substrate-induced respiration rate in two German loamy sand soils treated with metazachlor and dinoterb compared to the non-treated control soils whereas the inhibiting effect of dinoterb was significantly stronger than that of metazachlor during the incubation time. Jones and Ananyeva (2001) determined positive correlations between pesticide transformation rates of the herbicide propachlor and of the fungicide metalaxyl and the microbial respiration activity such as basal and substrate-induced respiration as well as soil microbial biomass in sandy loamy Ultisols under forest, pasture and arable land. The authors stated that the occurrence of a relationship between the above mentioned variables may be a useful tool for evaluating and predicting the fate of pesticides and also the microbes in different soils. Consequently, the negative correlation between the metabolic quotient and pesticide transformation rates reflects the physiological status of the microbial community and may be used as an indicator of ecosystem stress since disturbance or stress of a microbial community affected by pesticide application leads to an increase of the metabolic quotient values (Jones and Ananyeva, 2001). Smith *et al*. (2000) investigated potential non-target effects of long-term application of the fungicide benomyl on microbial properties in Udic Argiustolls. They found a 10 % increase of substrate-induced respiration and distinct increases in microbial carbon content. By contrast, in a laboratory incubation of a silt-loam Luvisol Chen *et al*. (2001b) initially found a 30–50 % suppression of the substrate-induced respiration by application of benomyl, captan and chlorothalonil. In a laboratory study with three different soils types – Calcaric Phaeozem, Eutric Cambisol and Dystric Lithosol – contaminated with four levels of heavy metals (control, 300 mg kg^{-1} Zn, 100 mg kg^{-1} Cu, 50 mg kg^{-1} Ni, 50 mg kg^{-1} V and 3 mg kg^{-1} Cd as well as two- and threefold contents) Kandeler *et al*. (1996) detected more strongly decreasing values of soil microbial biomass content and basal respiration rate corresponding to increasing contamination levels.

Table 17a: Average respiratory parameter values in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Basal respiration [mg CO_2 kg^{-1} h^{-1}]								
F	6.6 ± 0.1 a BCD	6.2 ± 0.2 a BC	7.1 ± 0.2 c D	5.8 ± 0.0 a AB	6.6 ± 0.5 a BCD	5.0 ± 0.5 a A	8.2 ± 0.1 d E	6.9 ± 0.5 cde CD
R	6.9 ± 0.4 a B	6.5 ± 0.3 a B	5.4 ± 0.3 ab A	5.3 ± 0.1 a A	5.4 ± 0.3 a A	5.3 ± 0.1 a A	6.1 ± 0.2 ab AB	5.4 ± 0.5 ab A
M			4.9 ± 0.5 a A	5.6 ± 0.7 a A	4.6 ± 0.2 a A	5.2 ± 0.5 a A	7.5 ± 0.2 cd B	5.4 ± 0.2 ab A
M Es			4.9 ± 0.5 a A	5.2 ± 0.5 a A	5.0 ± 0.2 a A	5.4 ± 0.3 a A	6.1 ± 0.7 ab A	5.6 ± 0.4 ab A
M Cl			4.9 ± 0.5 a A	5.3 ± 0.6 a A	5.3 ± 0.6 a A	4.9 ± 0.4 a A	5.7 ± 0.0 a A	6.0 ± 0.3 abc A
M Es Cl			4.9 ± 0.5 a A	5.2 ± 0.3 a A	4.8 ± 0.2 a A	5.1 ± 0.3 a A	6.0 ± 0.2 a A	5.2 ± 0.1 a A
H			6.0 ± 0.2 b BC	5.4 ± 0.3 a B	4.2 ± 0.1 a A	6.8 ± 0.1 b CD	7.1 ± 0.4 cd D	5.4 ± 0.3 a B
H Bt			6.0 ± 0.2 b A	5.7 ± 0.5 a A	5.2 ± 0.7 a A	7.2 ± 0.4 b A	6.1 ± 0.0 ab A	6.4 ± 0.3 bcde A
H Cu			6.0 ± 0.2 b BC	5.1 ± 0.2 a AB	4.9 ± 0.4 a A	6.9 ± 0.2 b DE	6.2 ± 0.2 ab CD	7.2 ± 0.3 de E
H Bt Cu			6.0 ± 0.2 b BC	5.9 ± 0.2 a B	4.9 ± 0.1 a A	6.8 ± 0.2 b C	6.8 ± 0.6 abc C	7.9 ± 0.1 e D
Substrate-induced respiration [mg CO_2 kg^{-1} h^{-1}]								
F	46.5 ± 1.2 a AB	52.6 ± 1.4 a CDE	57.6 ± 0.5 a DE	49.1 ± 2.6 a ABC	59.4 ± 1.7 a E	44.3 ± 2.2 a A	50.2 ± 1.4 a BC	56.0 ± 3.1 a DE
R	56.1 ± 1.5 b AB	57.6 ± 1.4 a AB	64.1 ± 2.0 b C	60.8 ± 1.1 b BC	64.1 ± 1.7 bc C	59.0 ± 1.7 a ABC	53.4 ± 3.5 ab A	58.1 ± 1.5 a AB
M			65.5 ± 1.1 b C	67.8 ± 0.8 cd C	66.3 ± 0.7 bcd C	72.0 ± 1.7 bc D	56.5 ± 0.2 b A	62.4 ± 0.5 a B
M Es			65.5 ± 1.1 b AB	67.8 ± 0.6 c B	66.0 ± 0.8 bcd AB	73.4 ± 1.0 bc C	67.6 ± 1.5 cd B	62.5 ± 2.5 a A
M Cl			65.5 ± 1.1 b B	69.4 ± 0.4 cd C	67.4 ± 0.1 def BC	75.1 ± 0.9 c D	68.1 ± 0.2 d C	62.5 ± 1.2 a A
M Es Cl			65.5 ± 1.1 b AB	70.4 ± 0.6 cd CD	68.9 ± 0.2 ef BC	73.6 ± 2.0 bc D	68.6 ± 0.5 d BC	64.5 ± 2.1 a A
H			66.0 ± 0.4 b BC	70.9 ± 1.4 d D	69.4 ± 1.0 f CD	70.0 ± 1.1 b CD	63.5 ± 1.7 cd B	58.1 ± 2.2 a A
H Bt			66.0 ± 0.4 b B	74.5 ± 1.3 e C	68.1 ± 0.1 def B	70.3 ± 2.0 b BC	66.3 ± 0.9 cd B	57.3 ± 2.6 a A
H Cu			66.0 ± 0.4 b BC	74.0 ± 1.0 e D	67.1 ± 1.2 cde C	72.8 ± 0.6 b D	64.2 ± 1.2 cd B	55.8 ± 0.5 a A
H Bt Cu			66.0 ± 0.4 b BC	75.1 ± 1.3 e D	63.8 ± 1.5 ab B	80.2 ± 0.8 d E	68.5 ± 1.0 cd C	59.5 ± 2.1 a A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 17b: Average respiratory parameter values in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Microbial biomass [g C_{mic} kg^{-1}]								
F	0.74 ± 0.09 a AB	0.83 ± 0.02 a AB	0.86 ± 0.02 a B	0.69 ± 0.07 a A	0.91 ± 0.07 a B	0.68 ± 0.04 a A	0.84 ± 0.06 a AB	1.24 ± 0.04 def C
R	0.90 ± 0.01 a A	0.86 ± 0.04 a A	0.99 ± 0.08 a A	0.88 ± 0.04 a A	0.94 ± 0.02 a A	0.95 ± 0.07 a A	0.98 ± 0.04 abc A	1.40 ± 0.11 f B
M			0.94 ± 0.09 a A	0.89 ± 0.14 a A	0.87 ± 0.04 a A	0.85 ± 0.07 a A	0.93 ± 0.07 ab A	1.26 ± 0.03 ef A
M Es			0.94 ± 0.09 a A	0.93 ± 0.09 a A	1.08 ± 0.07 a A	0.88 ± 0.08 a A	1.18 ± 0.04 d A	1.12 ± 0.09 bcde A
M Cl			0.94 ± 0.09 a AB	0.82 ± 0.06 a A	1.02 ± 0.03 a BC	0.93 ± 0.09 a AB	1.11 ± 0.03 cd BC	1.16 ± 0.05 cde C
M Es Cl			0.94 ± 0.09 a A	0.92 ± 0.05 a A	0.93 ± 0.02 a A	0.82 ± 0.05 a A	1.08 ± 0.00 bcd A	0.93 ± 0.03 a A
H			1.00 ± 0.07 a BC	0.98 ± 0.04 a BC	0.78 ± 0.06 a A	0.89 ± 0.05 a AB	1.12 ± 0.07 cd C	0.98 ± 0.04 ab BC
H Bt			1.00 ± 0.07 a A	0.96 ± 0.08 a A	1.01 ± 0.13 a A	1.06 ± 0.14 a A	1.18 ± 0.06 d A	1.08 ± 0.07 abcd A
H Cu			1.00 ± 0.07 a A	0.84 ± 0.05 a A	0.83 ± 0.02 a A	0.92 ± 0.05 a A	0.89 ± 0.07 a A	1.03 ± 0.04 abc A
H Bt Cu			1.00 ± 0.07 a A	0.93 ± 0.01 a A	0.90 ± 0.02 a A	0.96 ± 0.05 a A	0.90 ± 0.02 a A	1.15 ± 0.06 bcde B
Metabolic quotient [mg CO_2 (g C_{mic})$^{-1}$ h^{-1}]								
F	9.1 ± 1.0 a CD	7.5 ± 0.3 a BC	8.3 ± 0.4 c BCD	8.7 ± 0.9 b BCD	7.3 ± 0.1 c AB	7.4 ± 0.6 c BC	9.9 ± 0.9 d D	5.5 ± 0.2 bc A
R	7.7 ± 0.5 a C	7.6 ± 0.2 a C	5.5 ± 0.3 ab B	6.1 ± 0.3 a B	5.8 ± 0.4 b B	5.6 ± 0.3 a B	6.2 ± 0.4 ab B	3.9 ± 0.2 a A
M			5.2 ± 0.3 a AB	6.4 ± 0.6 a C	5.3 ± 0.3 ab ABC	6.2 ± 0.4 a BC	8.0 ± 0.4 c D	4.3 ± 0.2 a A
M Es			5.2 ± 0.3 a A	5.7 ± 0.0 a A	4.7 ± 0.4 a A	6.2 ± 0.4 a A	5.2 ± 0.5 a A	5.0 ± 0.2 b A
M Cl			5.2 ± 0.3 a A	6.6 ± 0.3 a B	5.2 ± 0.4 ab A	5.3 ± 0.3 a A	5.2 ± 0.2 a A	5.2 ± 0.1 b A
M Es Cl			5.2 ± 0.3 a A	5.6 ± 0.1 a A	5.2 ± 0.2 ab A	6.2 ± 0.1 a B	5.6 ± 0.2 a A	5.6 ± 0.1 c A
H			6.0 ± 0.3 b A	5.5 ± 0.1 a A	5.4 ± 0.6 ab A	7.7 ± 0.5 c B	6.3 ± 0.1 ab A	5.5 ± 0.3 bc A
H Bt			6.0 ± 0.3 b AB	5.9 ± 0.3 a AB	5.1 ± 0.3 ab A	6.9 ± 0.5 bc B	5.2 ± 0.3 a A	5.9 ± 0.3 c AB
H Cu			6.0 ± 0.3 b A	6.2 ± 0.2 a AB	5.9 ± 0.3 b A	7.4 ± 0.3 c C	7.0 ± 0.4 bc BC	6.9 ± 0.2 d BC
H Bt Cu			6.0 ± 0.3 b AB	6.4 ± 0.1 a ABC	5.5 ± 0.0 ab A	7.1 ± 0.4 bc CD	7.5 ± 0.6 bc D	6.9 ± 0.3 d BCD

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 18a: Average respiratory parameter values subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Basal respiration [mg CO_2 kg^{-1} h^{-1}]								
F	–0.4 ± 0.1 a A	–0.3 ± 0.2 a A	1.7 ± 0.2 c C	0.5 ± 0.0 a AB	1.2 ± 0.5 a BC	–0.3 ± 0.5 a A	2.1 ± 0.1 d C	1.5 ± 0.5 cde C
R	0.0 ± 0.4 a A	0.0 ± 0.3 a A	0.0 ± 0.3 ab A	0.0 ± 0.1 a A	0.0 ± 0.3 a A	0.0 ± 0.1 a A	0.0 ± 0.2 ab A	0.0 ± 0.5 ab A
M			–0.6 ± 0.5 a A	0.3 ± 0.7 a AB	–0.9 ± 0.2 a A	–0.1 ± 0.5 a A	1.4 ± 0.2 cd B	0.0 ± 0.2 ab A
M Es			–0.6 ± 0.5 a A	–0.1 ± 0.5 a A	–0.5 ± 0.2 a A	0.1 ± 0.3 a A	0.0 ± 0.7 ab A	0.1 ± 0.4 ab A
M Cl			–0.6 ± 0.5 a A	0.1 ± 0.6 a A	–0.1 ± 0.6 a A	–0.4 ± 0.4 a A	–0.4 ± 0.0 a A	0.6 ± 0.3 abc A
M Es Cl			–0.6 ± 0.5 a A	–0.1 ± 0.3 a A	–0.6 ± 0.2 a A	–0.2 ± 0.3 a A	–0.1 ± 0.2 a A	–0.2 ± 0.1 a A
H			0.5 ± 0.2 b BC	0.1 ± 0.3 a B	–1.2 ± 0.1 a A	1.4 ± 0.1 b D	1.0 ± 0.4 cd CD	–0.1 ± 0.3 a B
H Bt			0.5 ± 0.2 b A	0.4 ± 0.5 a A	–0.2 ± 0.7 a A	1.8 ± 0.4 b A	0.0 ± 0.0 ab A	1.0 ± 0.3 bcde A
H Cu			0.5 ± 0.2 b B	–0.2 ± 0.2 a AB	–0.6 ± 0.4 a A	1.5 ± 0.2 b C	0.1 ± 0.2 ab AB	1.7 ± 0.3 de C
H Bt Cu			0.5 ± 0.2 b B	0.6 ± 0.2 a BC	–0.5 ± 0.1 a A	1.4 ± 0.2 b C	0.7 ± 0.6 abc BC	2.4 ± 0.1 e D
Substrate-induced respiration [mg CO_2 kg^{-1} h^{-1}]								
F	–9.6 ± 1.2 a ABC	–5.1 ± 1.4 a CD	–6.5 ± 0.5 a BCD	–11.7 ± 2.6 a AB	–4.7 ± 1.7 a CD	–14.7 ± 2.2 a A	–3.2 ± 1.4 a D	–2.1 ± 3.1 a D
R	0.0 ± 1.5 b A	0.0 ± 1.4 a A	0.0 ± 2.0 b A	0.0 ± 1.1 b A	0.0 ± 1.7 bc A	0.0 ± 1.7 a A	0.0 ± 3.5 ab A	0.0 ± 1.5 a A
M			1.4 ± 1.1 b AC	7.0 ± 0.8 cd CD	2.2 ± 0.7 bcd AB	13.1 ± 1.7 bc D	3.2 ± 0.2 b ABC	4.3 ± 0.5 a BC
M Es			1.4 ± 1.1 b A	7.0 ± 0.6 c B	1.9 ± 0.8 bcd A	14.4 ± 1.0 bc C	14.2 ± 1.5 cd C	4.4 ± 2.5 a AB
M Cl			1.4 ± 1.1 b A	8.6 ± 0.4 cd C	3.3 ± 0.1 def AB	16.1 ± 0.9 c D	14.7 ± 0.2 d D	4.5 ± 1.2 a B
M Es Cl			1.4 ± 1.1 b A	9.6 ± 0.6 cd C	4.8 ± 0.2 ef AB	14.7 ± 2.0 bc D	15.2 ± 0.5 d D	6.4 ± 2.1 a BC
H			1.9 ± 0.4 b AB	10.1 ± 1.4 d C	5.3 ± 1.0 f B	11.1 ± 1.1 b C	10.1 ± 1.7 cd C	0.1 ± 2.2 a A
H Bt			1.9 ± 0.4 b AB	13.7 ± 1.3 e C	4.0 ± 0.1 def B	11.3 ± 2.0 b C	12.9 ± 0.9 cd C	–0.7 ± 2.6 a A
H Cu			1.9 ± 0.4 b B	13.2 ± 1.0 e C	3.0 ± 1.2 cde B	13.8 ± 0.6 b C	10.8 ± 1.2 cd C	–2.3 ± 0.5 a A
H Bt Cu			1.9 ± 0.4 b A	14.4 ± 1.3 e B	–0.3 ± 1.5 ab A	21.2 ± 0.8 d B	15.1 ± 1.0 cd B	1.4 ± 2.1 a A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 18b: Average respiratory parameter values subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application			after pesticide application		
Microbial biomass [g C_{mic} kg^{-1}]								
F	–0.16 ± 0.09 a A	–0.03 ± 0.02 a A	–0.13 ± 0.02 a A	–0.19 ± 0.07 a A	–0.03 ± 0.07 a A	–0.28 ± 0.04 a A	–0.14 ± 0.06 a A	–0.16 ± 0.04 def A
R	0.00 ± 0.01 a A	0.00 ± 0.04 a A	0.00 ± 0.08 a A	0.00 ± 0.04 a A	0.00 ± 0.02 a A	0.00 ± 0.07 a A	0.00 ± 0.04 abc A	0.00 ± 0.11 f A
M			–0.05 ± 0.09 a A	0.09 ± 0.14 a A	–0.07 ± 0.04 a A	0.11 ± 0.07 a A	–0.05 ± 0.07 ab A	–0.14 ± 0.03 ef A
M Es			–0.05 ± 0.09 a ABC	0.05 ± 0.09 a BC	0.14 ± 0.07 a BC	–0.08 ± 0.08 a AB	0.20 ± 0.04 d C	–0.27 ± 0.09 bcde A
M Cl			–0.05 ± 0.09 a AB	–0.06 ± 0.06 a AB	0.08 ± 0.03 a B	–0.02 ± 0.09 a B	0.13 ± 0.03 cd B	–0.24 ± 0.05 cde A
M Es Cl			–0.05 ± 0.09 a BC	0.05 ± 0.05 a C	–0.01 ± 0.02 a BC	–0.13 ± 0.05 a B	0.10 ± 0.00 bcd C	–0.47 ± 0.03 a A
H			0.01 ± 0.07 a BC	0.11 ± 0.04 a C	–0.16 ± 0.06 a B	–0.07 ± 0.05 a B	0.14 ± 0.07 cd C	–0.42 ± 0.04 ab A
H Bt			0.01 ± 0.07 a B	0.09 ± 0.08 a B	0.07 ± 0.13 a B	0.11 ± 0.14 a B	0.20 ± 0.06 d B	–0.32 ± 0.07 abcd A
H Cu			0.01 ± 0.07 a B	–0.04 ± 0.05 a B	–0.11 ± 0.02 a B	–0.03 ± 0.05 a B	–0.09 ± 0.07 a B	–0.37 ± 0.04 abc A
H Bt Cu			0.01 ± 0.07 a B	0.05 ± 0.01 a B	–0.04 ± 0.02 a B	0.01 ± 0.05 a B	–0.08 ± 0.02 a B	–0.25 ± 0.06 bcde A
Metabolic quotient [mg CO_2 (g C_{mic})$^{-1}$ h^{-1}]								
F	1.4 ± 1.0 a AB	–0.1 ± 0.3 a A	2.8 ± 0.4 c BC	2.5 ± 0.9 b BC	1.5 ± 0.1 c AB	1.8 ± 0.6 c AB	3.7 ± 0.9 d C	1.7 ± 0.2 bc AB
R	0.0 ± 0.5 a A	0.0 ± 0.2 a A	0.0 ± 0.3 ab A	0.0 ± 0.3 a A	0.0 ± 0.4 b A	0.0 ± 0.3 a A	0.0 ± 0.4 ab A	0.0 ± 0.2 a A
M			–0.4 ± 0.3 a A	0.3 ± 0.6 a A	–0.5 ± 0.3 ab A	0.5 ± 0.4 a A	1.8 ± 0.4 c B	0.4 ± 0.2 a A
M Es			–0.4 ± 0.3 a AB	–0.5 ± 0.0 a AB	–1.1 ± 0.4 a A	0.6 ± 0.4 a BC	–1.1 ± 0.5 a A	1.1 ± 0.2 b C
M Cl			–0.4 ± 0.3 a AB	0.5 ± 0.3 a B	–0.6 ± 0.4 ab A	–0.3 ± 0.3 a AB	–1.1 ± 0.2 a A	1.3 ± 0.1 b C
M Es Cl			–0.4 ± 0.3 a AB	–0.5 ± 0.1 a A	–0.6 ± 0.2 ab A	0.6 ± 0.1 a BC	–0.7 ± 0.2 a A	1.7 ± 0.1 c C
H			0.5 ± 0.3 b A	–0.6 ± 0.1 a A	–0.3 ± 0.6 ab A	2.1 ± 0.5 c B	0.1 ± 0.1 ab A	1.6 ± 0.3 bc B
H Bt			0.5 ± 0.3 b BC	–0.2 ± 0.3 a AB	–0.7 ± 0.3 ab A	1.2 ± 0.5 bc CD	–1.1 ± 0.3 a A	2.0 ± 0.3 c D
H Cu			0.5 ± 0.3 b A	0.0 ± 0.2 a A	0.1 ± 0.3 b A	1.8 ± 0.3 c B	0.8 ± 0.4 bc A	3.0 ± 0.2 d C
H Bt Cu			0.5 ± 0.3 b AB	0.3 ± 0.1 a A	–0.3 ± 0.0 ab A	1.4 ± 0.4 bc B	1.3 ± 0.6 bc B	3.0 ± 0.3 d C

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

3.2.3.3 Nitrogen parameters

The net nitrogen mineralisation rate ranged between 0.7 and 2.7 mg mineral nitrogen kg^{-1} d^{-1} (Table 19) during the field experiment. The values of the fallow plots showed the same trend than those of the reference ones, but they were slightly lower than the latter. The conventional treatments only provided reliable data four and nine weeks after pesticide application. Due to a high ammonium content (data not shown) because of mineral nitrogen fertilisation extraordinarily varying data were obtained not suitable for evaluation. Hence, these samples were designated as not detectable in Table 19 and 20. Nevertheless, the net nitrogen mineralisation rate of the conventionally managed plots of the last two sampling days was higher than that of the organically managed ones being significant ($P < 0.05$) nine weeks after pesticide application. This was attributed to the mineral fertilisation through which the mineralisation was activated and released further mineral nitrogen. The net nitrogen mineralisation rate of the organic treatments fluctuated strongly throughout the field experiment duration and was slightly higher at the end of the experiment than at the beginning both with or without the subtraction of the reference values. In both management systems the pesticide application promoted the net nitrogen mineralisation since the plots only treated with the respective fertiliser showed lower values compared to those treated with pesticides.

The net nitrification rate ranged between 1.2 and 5.9 mg nitrate nitrogen kg^{-1} h^{-1} (Table 19) during the field experiment. The fallow plots having the lowest values showed a similar trend compared to the reference ones. After strong variations, the values of the organic treatments returned to those of the initial state, which was equal to the values subtracting those of the reference plots. By contrast, both with or without the subtraction of the reference plots values the net nitrification rate of the conventional treatments exhibited continuously decreasing values from the beginning to the end of the experiment. This fact was caused by a highly significant ($P < 0.01$) correlation between net nitrification and ammonium content ($R = 0.755$) since via mineral nitrogen fertilisation a high amount of ammonium was added to the soil stimulating net nitrification and the fewer the ammonium content became over time the fewer nitrate could be produced. The same result was obtained for the values subtracting those of the reference plots and the significantly ($P < 0.05$) lower net nitrification rate of the organic in comparison to the conventional treatments was again explained by the different

fertiliser and thus ammonium application. Concerning pesticide applications no effect could be detected in the conventional treatments, while in the organic treatments the application promoted the net nitrification as the plots only treated with humus exhibited lower values compared to those treated with pesticides.

The potential denitrification rate ranged between 0.4 and 1.3 mg nitrous oxide kg^{-1} h^{-1} (Table 19) during the field experiment. The values of the fallow treatments were higher at the beginning and lower at the end of the experiment than those of the reference ones and showed a different development compared to the latter. Two weeks after the experiment beginning there was a distinct maximum in potential denitrification in soils of all treatments in consequence of the precipitation event (Figure 16, page 78) leading to anaerobic soil conditions. The potential denitrification rate of the conventional treatments with and without the subtraction of the reference values varied after the beginning of the field experiment and was significantly ($P < 0.05$) lower at the end. The values of the organic treatments were significantly ($P < 0.05$) higher at the end of the experiment than at the beginning, while the values subtracting those of the reference plots were only tendentially higher at the end. The potential denitrification rate of the conventional treatments was significantly ($P < 0.05$) lower than that of the organic ones, which could be explained the by different fertilisation. The potential denitrification rate exhibited highly significant ($P < 0.01$) correlations with total organic carbon ($R = 0.424$) and nitrogen ($R = 0.395$) contents as well as with the pH value ($R = 0.483$). By means of humus application easily degradable organic matter was added to the organic treatments, while via mineral sulfate and phosphate addition to the conventional treatments the pH value was reduced (Figure 17, page 79) below the pH optimum for the potential denitrification, which was examined being pH 7 – 8 by Šimek and Hopkins (1999) for long-term organically and minerally fertilised arable soils. Since pH value, total organic carbon and nitrogen contents were significantly ($P < 0.05$) higher in soils of the organically managed plots compared to the conventionally managed ones the potential denitrification heavily depending on these conditions, exhibited a higher rate in the organic treatments. However, with respect to the pesticide applications no effects could be detected in both management systems.

Table 19a: Average nitrogen parameter values in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Net nitrogen mineralisation [mg N_{min} kg^{-1} d^{-1}]								
F	1.3 ± 0.0 a A	1.3 ± 0.0 a A	1.4 ± 0.3 a A	0.7 ± 0.1 a A	1.3 ± 0.2 a A	0.9 ± 0.1 a A	0.8 ± 0.1 a A	1.3 ± 0.2 a A
R	1.4 ± 0.2 a B	1.2 ± 0.1 a B	1.5 ± 0.1 a B	0.7 ± 0.2 a A	1.5 ± 0.1 a B	0.8 ± 0.1 a A	1.2 ± 0.1 a B	1.5 ± 0.1 ab B
M			n. d.	n. d.	n. d.	n. d.	1.4 ± 0.7 a A	2.2 ± 0.2 de A
M Es			n. d.	n. d.	n. d.	n. d.	1.6 ± 0.2 a A	2.3 ± 0.2 de A
M Cl			n. d.	n. d.	n. d.	n. d.	1.9 ± 0.4 a A	2.3 ± 0.2 e A
M Es Cl			n. d.	n. d.	n. d.	n. d.	1.5 ± 0.2 a A	2.1 ± 0.2 cde A
H			0.9 ± 0.1 a A	1.4 ± 0.2 b A	1.2 ± 0.1 a A	1.1 ± 0.2 a A	1.3 ± 0.1 a A	1.4 ± 0.1 ab A
H Bt			0.9 ± 0.1 a A	2.1 ± 0.1 c C	1.5 ± 0.1 a B	2.5 ± 0.2 c C	1.4 ± 0.1 a B	1.7 ± 0.1 abc B
H Cu			0.9 ± 0.1 a A	2.4 ± 0.1 cd E	1.3 ± 0.2 a BC	1.9 ± 0.1 b D	1.3 ± 0.1 a AB	1.7 ± 0.1 abc CD
H Bt Cu			0.9 ± 0.1 a A	2.7 ± 0.1 d C	1.3 ± 0.2 a A	2.5 ± 0.1 c C	1.2 ± 0.1 a A	1.9 ± 0.2 bcd B
Net nitrification [mg NO_3^--N kg^{-1} d^{-1}]								
F	1.9 ± 0.0 a D	1.8 ± 0.0 a BCD	1.9 ± 0.3 a D	1.2 ± 0.1 a A	1.8 ± 0.2 a CD	1.5 ± 0.1 a ABCD	1.3 ± 0.1 a AB	1.4 ± 0.1 a ABC
R	2.3 ± 0.2 a B	2.0 ± 0.1 a AB	2.4 ± 0.2 a B	1.7 ± 0.1 b A	2.5 ± 0.1 a B	2.1 ± 0.2 b AB	2.0 ± 0.2 a AB	1.7 ± 0.1 abc A
M			5.1 ± 0.3 b B	5.1 ± 0.3 e B	5.6 ± 0.3 b B	5.0 ± 0.4 d B	4.6 ± 0.7 b B	2.8 ± 0.2 d A
M Es			5.1 ± 0.3 b BC	5.6 ± 0.6 e BC	5.9 ± 0.4 b C	5.0 ± 0.5 d BC	4.3 ± 0.5 b AB	3.1 ± 0.4 d A
M Cl			5.1 ± 0.3 b BC	5.5 ± 0.4 e C	5.0 ± 0.1 b BC	5.6 ± 0.5 d C	4.3 ± 0.3 b AB	2.9 ± 0.3 d A
M Es Cl			5.1 ± 0.3 b B	5.2 ± 0.3 e B	5.4 ± 0.4 b B	4.8 ± 0.3 d B	4.3 ± 0.5 b B	2.8 ± 0.4 d A
H			1.9 ± 0.1 a B	2.2 ± 0.1 bc C	2.0 ± 0.1 a BC	2.0 ± 0.1 ab BC	1.9 ± 0.1 a B	1.5 ± 0.1 ab A
H Bt			1.9 ± 0.1 a A	2.8 ± 0.0 cd C	2.5 ± 0.3 a BC	2.9 ± 0.2 c C	2.1 ± 0.1 a AB	1.8 ± 0.2 bc A
H Cu			1.9 ± 0.1 a A	2.8 ± 0.2 cd C	2.3 ± 0.2 a B	2.4 ± 0.1 bc BC	1.8 ± 0.1 a A	1.8 ± 0.1 bc A
H Bt Cu			1.9 ± 0.1 a A	3.0 ± 0.1 d B	2.2 ± 0.3 a A	3.0 ± 0.1 c B	1.8 ± 0.1 a A	1.9 ± 0.2 c A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride; n. d.: not detectable.

Table 19b: Average nitrogen parameter values in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Potential denitrification (N_2O emission) [mg N_2O kg^{-1} h^{-1}]								
F	0.52 ± 0.02 a A	0.62 ± 0.04 a AB	0.66 ± 0.05 a AB	0.87 ± 0.07 a C	0.72 ± 0.04 a BC	0.62 ± 0.08 a AB	0.70 ± 0.07 abcd BC	0.73 ± 0.06 ab BC
R	0.55 ± 0.02 a A	0.55 ± 0.08 a A	0.56 ± 0.10 a A	0.67 ± 0.07 a A	0.59 ± 0.05 a A	1.08 ± 0.16 a A	0.79 ± 0.23 cd A	0.86 ± 0.15 bc A
M			0.75 ± 0.09 a C	0.55 ± 0.01 a B	0.62 ± 0.03 a BC	0.83 ± 0.11 a C	0.41 ± 0.03 a A	0.50 ± 0.02 a B
M Es			0.75 ± 0.09 a A	0.60 ± 0.08 a A	0.71 ± 0.05 a A	0.86 ± 0.12 a A	0.52 ± 0.09 ab A	0.49 ± 0.03 a A
M Cl			0.75 ± 0.09 a BC	0.57 ± 0.06 a AB	0.80 ± 0.09 a C	1.13 ± 0.06 a D	0.42 ± 0.03 a A	0.55 ± 0.02 a AB
M Es Cl			0.75 ± 0.09 a C	0.62 ± 0.03 a BC	0.68 ± 0.04 a C	1.05 ± 0.03 a D	0.46 ± 0.05 ab A	0.52 ± 0.01 a AB
H			0.69 ± 0.01 a BC	0.61 ± 0.05 a AB	0.47 ± 0.05 a A	0.85 ± 0.04 a CD	0.87 ± 0.11 d CD	1.02 ± 0.10 c D
H Bt			0.69 ± 0.01 a A	0.61 ± 0.07 a A	0.70 ± 0.14 a A	1.08 ± 0.22 a A	0.88 ± 0.08 d A	1.06 ± 0.11 cd A
H Cu			0.69 ± 0.01 a A	0.63 ± 0.05 a A	0.55 ± 0.05 a A	1.00 ± 0.11 a B	0.73 ± 0.09 bcd A	1.01 ± 0.09 c B
H Bt Cu			0.69 ± 0.01 a A	0.75 ± 0.12 a A	0.70 ± 0.06 a A	1.13 ± 0.27 a B	0.80 ± 0.05 cd AB	1.30 ± 0.14 d B
Potential denitrification (CO_2 emission) [mg CO_2 kg^{-1} h^{-1}]								
F	6.4 ± 0.4 a A	6.6 ± 0.3 a A	6.2 ± 0.2 a A	6.6 ± 0.3 a A	10.0 ± 0.5 abc B	13.8 ± 0.9 c B	12.1 ± 0.9 cd B	8.2 ± 0.0 a B
R	7.1 ± 0.2 a B	7.1 ± 0.2 a B	6.6 ± 0.5 a AB	6.2 ± 0.4 a A	10.5 ± 0.8 bcd C	16.5 ± 0.4 d D	9.7 ± 0.1 b C	9.0 ± 0.7 ab C
M			8.8 ± 0.6 b B	6.5 ± 0.6 a A	8.6 ± 0.4 a B	16.6 ± 0.4 d C	8.2 ± 0.5 a B	8.3 ± 0.7 a B
M Es			8.8 ± 0.6 b AB	7.8 ± 0.7 a A	9.8 ± 0.5 abc B	17.6 ± 0.9 d C	9.5 ± 0.4 ab B	8.8 ± 0.6 ab AB
M Cl			8.8 ± 0.6 b A	7.6 ± 0.4 a A	9.3 ± 0.5 ab A	12.9 ± 2.0 bc B	9.0 ± 0.5 ab A	9.3 ± 0.6 abc A
M Es Cl			8.8 ± 0.6 b B	7.0 ± 0.1 a A	10.8 ± 0.2 bcde CD	10.3 ± 0.4 a C	11.6 ± 0.3 d D	8.1 ± 0.2 a B
H			9.2 ± 0.2 b AB	8.0 ± 0.5 a AB	11.6 ± 0.9 de C	9.3 ± 0.5 a AB	12.7 ± 0.4 d C	9.7 ± 0.4 bc B
H Bt			9.2 ± 0.2 b B	6.9 ± 0.5 a A	10.4 ± 0.2 bcd C	10.5 ± 0.3 ab C	12.7 ± 0.5 d D	8.9 ± 0.3 ab B
H Cu			9.2 ± 0.2 b B	7.0 ± 0.0 a A	11.0 ± 0.4 cde DE	10.6 ± 0.3 ab CD	11.5 ± 0.4 d E	10.1 ± 0.2 bc C
H Bt Cu			9.2 ± 0.2 b B	7.6 ± 0.3 a A	12.2 ± 0.6 e E	10.6 ± 0.6 ab CD	10.1 ± 0.2 bc BC	10.7 ± 0.4 c D

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 20a: Average nitrogen parameter values subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Net nitrogen mineralisation [mg N_{min} kg^{-1} d^{-1}]								
F	–0.2 ± 0.0 a A	0.1 ± 0.0 a A	–0.1 ± 0.3 a A	0.0 ± 0.1 a A	–0.2 ± 0.2 a A	0.1 ± 0.1 a A	–0.4 ± 0.1 a A	–0.2 ± 0.2 a A
R	0.0 ± 0.2 a A	0.0 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.2 a A	0.0 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.1 ab A
M			n. d.	n. d.	n. d.	n. d.	0.2 ± 0.7 a A	0.7 ± 0.2 de A
M Es			n. d.	n. d.	n. d.	n. d.	0.4 ± 0.2 a A	0.8 ± 0.2 de A
M Cl			n. d.	n. d.	n. d.	n. d.	0.7 ± 0.4 a A	0.9 ± 0.2 e A
M Es Cl			n. d.	n. d.	n. d.	n. d.	0.3 ± 0.2 a A	0.6 ± 0.2 cde A
H			–0.5 ± 0.1 a A	0.6 ± 0.2 b D	–0.2 ± 0.1 a AB	0.3 ± 0.2 a CD	0.0 ± 0.1 a BC	–0.1 ± 0.1 ab BC
H Bt			–0.5 ± 0.1 a A	1.4 ± 0.1 c C	0.0 ± 0.1 a B	1.7 ± 0.2 c C	0.2 ± 0.1 a B	0.2 ± 0.1 abc B
H Cu			–0.5 ± 0.1 a A	1.6 ± 0.1 cd C	–0.1 ± 0.2 a B	1.1 ± 0.1 b C	0.0 ± 0.1 a B	0.2 ± 0.1 abc B
H Bt Cu			–0.5 ± 0.1 a A	2.0 ± 0.1 d D	–0.2 ± 0.2 a B	1.7 ± 0.1 c D	0.0 ± 0.1 a BC	0.4 ± 0.2 bcd CD
Net nitrification [mg NO_3^--N kg^{-1} d^{-1}]								
F	–0.4 ± 0.0 a A	–0.3 ± 0.0 a A	–0.5 ± 0.3 a A	–0.5 ± 0.1 a A	–0.6 ± 0.2 a A	–0.6 ± 0.1 a A	–0.7 ± 0.1 a A	–0.2 ± 0.1 a A
R	0.0 ± 0.2 a A	0.0 ± 0.1 a A	0.0 ± 0.2 a A	0.0 ± 0.1 b A	0.0 ± 0.1 a A	0.0 ± 0.2 b A	0.0 ± 0.2 a A	0.0 ± 0.1 abc A
M			2.7 ± 0.3 b B	3.3 ± 0.3 e B	3.2 ± 0.3 b B	2.9 ± 0.4 d B	2.6 ± 0.7 b B	1.1 ± 0.2 d A
M Es			2.7 ± 0.3 b ABC	3.9 ± 0.6 e C	3.4 ± 0.4 b BC	2.9 ± 0.5 d BC	2.3 ± 0.5 b AB	1.4 ± 0.4 d A
M Cl			2.7 ± 0.3 b BC	3.7 ± 0.4 e C	2.5 ± 0.1 b B	3.5 ± 0.5 d C	2.2 ± 0.3 b AB	1.3 ± 0.3 d A
M Es Cl			2.7 ± 0.3 b B	3.4 ± 0.3 e B	2.9 ± 0.4 b B	2.7 ± 0.3 d B	2.3 ± 0.5 b AB	1.1 ± 0.4 d A
H			–0.6 ± 0.1 a A	0.5 ± 0.1 bc D	–0.4 ± 0.1 a AB	–0.1 ± 0.1 ab C	–0.2 ± 0.1 a BC	–0.2 ± 0.1 ab BC
H Bt			–0.6 ± 0.1 a A	1.1 ± 0.0 cd C	0.1 ± 0.3 a B	0.8 ± 0.2 c C	0.1 ± 0.1 a B	0.1 ± 0.2 bc B
H Cu			–0.6 ± 0.1 a A	1.1 ± 0.2 cd D	–0.1 ± 0.2 a ABC	0.3 ± 0.1 bc C	–0.3 ± 0.1 a AB	0.1 ± 0.1 bc BC
H Bt Cu			–0.6 ± 0.1 a A	1.3 ± 0.1 d C	–0.2 ± 0.3 a AB	0.9 ± 0.1 c C	–0.3 ± 0.1 a AB	0.2 ± 0.2 c B

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride; n. d.: not detectable.

Table 20b: Average nitrogen parameter values subtracting reference means in soils of all treatments during the experiment with standard errors.

Parameters	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Potential denitrification (N_2O emission) [mg N_2O kg^{-1} h^{-1}]								
F	0.00 ± 0.02 a BCD	0.01 ± 0.04 a CDE	0.01 ± 0.05 a DE	0.20 ± 0.07 a E	0.13 ± 0.04 a E	–0.46 ± 0.08 a A	–0.10 ± 0.07 abcd BC	–0.13 ± 0.06 ab B
R	0.00 ± 0.02 a A	0.00 ± 0.08 a A	0.00 ± 0.10 a A	0.00 ± 0.07 a A	0.00 ± 0.05 a A	0.00 ± 0.16 a A	0.00 ± 0.23 cd A	0.00 ± 0.15 bc A
M			0.19 ± 0.09 a D	–0.13 ± 0.01 a BC	0.03 ± 0.03 a CD	–0.25 ± 0.11 a AB	–0.39 ± 0.03 a A	–0.36 ± 0.02 a A
M Es			0.19 ± 0.09 a D	–0.07 ± 0.08 a BC	0.12 ± 0.05 a CD	–0.22 ± 0.12 a AB	–0.27 ± 0.09 ab AB	–0.37 ± 0.03 a A
M Cl			0.19 ± 0.09 a C	–0.10 ± 0.06 a B	0.21 ± 0.09 a C	0.05 ± 0.06 a BC	–0.37 ± 0.03 a A	–0.30 ± 0.02 a A
M Es Cl			0.19 ± 0.09 a C	–0.06 ± 0.03 a B	0.09 ± 0.04 a BC	–0.03 ± 0.03 a B	–0.33 ± 0.05 ab A	–0.33 ± 0.01 a A
H			0.14 ± 0.01 a CD	–0.07 ± 0.05 a ABC	–0.21 ± 0.05 a AB	–0.23 ± 0.04 a A	0.07 ± 0.11 d BCD	0.17 ± 0.10 c D
H Bt			0.14 ± 0.01 a A	–0.06 ± 0.07 a A	0.11 ± 0.14 a A	0.00 ± 0.22 a A	0.09 ± 0.08 d A	0.20 ± 0.11 cd A
H Cu			0.14 ± 0.01 a A	–0.04 ± 0.05 a A	–0.04 ± 0.05 a A	–0.08 ± 0.11 a A	–0.06 ± 0.09 bcd A	0.16 ± 0.09 c A
H Bt Cu			0.14 ± 0.01 a A	0.08 ± 0.12 a A	0.11 ± 0.06 a A	0.05 ± 0.27 a A	0.00 ± 0.05 cd A	0.44 ± 0.14 d A
Potential denitrification (CO_2 emission) [mg CO_2 kg^{-1} h^{-1}]								
F	–0.7 ± 0.4 a B	–0.5 ± 0.3 a B	–0.5 ± 0.2 a B	0.4 ± 0.3 a B	–0.5 ± 0.5 abc B	–2.7 ± 0.9 c A	2.4 ± 0.9 cd C	–0.9 ± 0.0 a B
R	0.0 ± 0.2 a A	0.0 ± 0.2 a A	0.0 ± 0.5 a A	0.0 ± 0.4 a A	0.0 ± 0.8 bcd A	0.0 ± 0.4 d A	0.0 ± 0.1 b A	0.0 ± 0.7 ab A
M			2.2 ± 0.6 b D	0.3 ± 0.6 a C	–1.9 ± 0.4 a A	0.1 ± 0.4 d BC	–1.4 ± 0.5 a AB	–0.7 ± 0.7 a ABC
M Es			2.2 ± 0.6 b C	1.6 ± 0.7 a BC	–0.6 ± 0.5 abc A	1.1 ± 0.9 d ABC	–0.2 ± 0.4 ab AB	–0.2 ± 0.6 ab AB
M Cl			2.2 ± 0.6 b C	1.4 ± 0.4 a BC	–1.1 ± 0.5 ab AB	–3.6 ± 2.0 bc A	–0.7 ± 0.5 ab AB	0.3 ± 0.6 abc BC
M Es Cl			2.2 ± 0.6 b D	0.9 ± 0.1 a C	0.3 ± 0.2 bcde C	–6.2 ± 0.4 a A	1.9 ± 0.3 d D	–0.9 ± 0.2 a B
H			2.6 ± 0.2 b CD	1.9 ± 0.5 a BC	1.1 ± 0.9 de BC	–7.2 ± 0.5 a A	3.0 ± 0.4 d D	0.7 ± 0.4 bc B
H Bt			2.6 ± 0.2 b C	0.7 ± 0.5 a B	–0.1 ± 0.2 bcd B	–6.0 ± 0.3 ab A	3.0 ± 0.5 d C	–0.2 ± 0.3 ab B
H Cu			2.6 ± 0.2 b D	0.8 ± 0.0 a B	0.6 ± 0.4 cde B	–5.9 ± 0.3 ab A	1.9 ± 0.4 d C	1.1 ± 0.2 bc BC
H Bt Cu			2.6 ± 0.2 b D	1.4 ± 0.3 a BC	1.8 ± 0.6 e CD	–5.9 ± 0.6 ab A	0.5 ± 0.2 bc B	1.6 ± 0.4 c BC

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

The CO_2 emission rate of the potential denitrification ranged between 5.8 and 17.6 mg CO_2 kg^{-1} h^{-1} (Table 19) during the field experiment. The trend of the fallow plots values was similar but mostly lower in comparison to that of the reference ones. At the end of the experiment the CO_2 emission rate of the conventional and organic treatments approximated that at the beginning, while subtracting the CO_2 emission rate of the reference plots they showed tendentially decreasing values. At the beginning of the experiment the plots of both management systems showed a similar emission rate. Two weeks after pesticide application the CO_2 emission rate of the reference plots and of the plots of three conventional treatments raised significantly ($P < 0.05$) possibly because of the precipitation event (Figure 16, page 78). Four weeks after application until the experiment end the values of the organically managed plots were tendentially higher than those of the conventionally managed ones similar to the N_2O emission rate. The pesticide application had no effect on potential denitrification both in conventional and organic treatments.

Summarising, again the reported ranges of nitrogen parameters were in the same order of magnitude than those of the land use system samples and thus, they also can be considered typical for Mollisols under agriculture at least in Argentina. The fallow and reference plots showed very similar trends for all nitrogen parameters. Due to a higher ammonium content because of mineral fertilisation the nitrogen mineralisation activities were significantly ($P < 0.05$) higher in the conventional treatments compared to the organic ones. By contrast, the organically managed plots exhibited a markedly higher potential denitrification activity than the conventionally managed ones caused by organic matter addition through humus application. With regard to pesticide as well as heavy metal applications to the soils only sparsely effects on soil microbial community functions could be detected, while in literature several impacts of pesticides on these parameters were reported.

Banerjee and Dey (1992) found a significant increase in net nitrogen mineralisation in the rhizosphere microflora of Gangetic alluviums treated with a combination of the herbicide basalin and either the fungicide dithane or the fungicide bengard when compared to control soils in a random block design field experiment in West Bengal, India. However, when the pesticides were applied alone the authors reported a decrease in net nitrogen mineralisation. Chen *et al*. (2001b) detected a significantly increased total inorganic nitrogen content due to higher rates of net nitrogen mineralisation and net nitrification in silt-loam Luvisols

incubated with the herbicides benomyl, captan and chlorothalonil caused by mineralisation of dead organisms. Beulke and Malkomes (2001) determined a higher rate of net nitrogen mineralisation in two German loamy sand soils treated with the herbicides metazachlor and dinoterb when compared to the non-treated control soils, which was confirmed by Engelen *et al*. (1998) who studied the impacts of the herbicides dinoterb and metamitron on a loamy sand and also found stimulating effects on the net nitrogen mineralisation. In a laboratory investigation on three diverse soil types (Calcaric Phaeozem, Eutric Cambisol and Dystric Lithosol) contaminated with four heavy metal levels (uncontaminated control, 300 mg kg^{-1} zinc, 100 mg kg^{-1} copper, 50 mg kg^{-1} nickel, 50 mg kg^{-1} vanadium and 3 mg kg^{-1} cadmium as well as two- and threefold concentrations of these heavy metals) Kandeler *et al*. (1996) determined a more strongly decreasing nitrogen mineralisation activity according to raising contamination levels.

3.2.3.4 Principal component analysis

As shown above, by means of individual soil microbial functional parameters it was hardly possible to distinguish between soils of different treatments, in particular different cultivation systems and primarily different plant protection practices. The latter fact might be caused by the limited bioavailability of the pesticides in soils of high clay and organic carbon content as explained by Ahtiainen *et al*. (2003) who found microbial activities and biomass following the weather conditions during the growing season whereas significant effects of pesticide treatments on microbial processes were not observed. Contradictory, in laboratory studies the toxicity of certain pesticides was clearly detected by bacterial toxicity tests, while in the field inhibitory effects were observed only at unrealistically high contents (Ahtiainen *et al*., 2003).

A principal component analysis (PCA) was applied in order to pool correlating data into independent components. For the analysed parameter values with the exception of those of the net nitrogen mineralisation because of lacking data (see 3.2.3.3, page 97) a Kaiser-Meyer-Olkin-measure of sampling adequacy of 0.521 was computed, which indicated that a PCA was useful for the data set, while a value lower than 0.0005 calculated from the Bartlett's Test of Sphericity showed that significant relationships among the analysed parameters existed

and that the data were suitable for a PCA (Backhaus and Erichson, 2003). Comparable to the PCA of the soil microbial functional parameters of the different land use systems (see 3.1.3.4, page 54) four similar principal components explaining 66.8 % of the total variance were extracted (Table 21) whereas in this PCA the cellulase activity could not be attributed to a principal component because of very low correlation coefficients and thus, it was considered possessing a too low contribution at the whole soil microbial community function.

Table 21: Varimax rotated (after Kaiser normalisation) component matrix of the principal component analysis of all investigated soil microbial functional parameters. Each component combines variables with the highest factor loadings (bold) in a column.

Microbial functional parameters	Component 1	Component 2	Component 3	Component 4	Interpretation
Microbial biomass	**0.84**	0.16	-0.06	0.15	Microbial capacity
Arylsulfatase activity	**0.80**	-0.22	0.19	0.01	
Acid phosphatase activity	**0.58**	-0.57	-0.06	0.26	
Dehydrogenase activity	0.11	**0.77**	0.11	-0.06	Mineralisation activity
Substrate-induced respiration	0.14	**0.62**	-0.41	0.30	
Net-nitrification	-0.10	**0.58**	-0.41	0.12	
Urease activity	0.23	**-0.60**	0.17	-0.05	
Basal respiration	0.32	-0.08	**0.85**	0.12	Metabolic activity
Metabolic quotient	-0.45	-0.26	**0.77**	-0.07	
Potential denitrification (CO_2)	-0.06	0.06	-0.10	**0.86**	Nitrogen trans-formation potential
Potential denitrification (N_2O)	0.42	-0.03	0.19	**0.67**	
Cellulase activity	-0.45	0.12	0.33	0.34	Not specific

The first component (microbial capacity) explaining 20.3 % of the total variance including microbial biomass as well as arylsulfatase and acid phosphatase activity (not highly specific due to similarly high correlation coefficients in two components) corresponded to the results of Taylor *et al*. (2002) who examined strong correlations between these three parameters in comparison of enzyme activities using various techniques. Consequently, this indicated the higher the microbial biomass content is the more enzymes are released into the soil in order to degrade large sulfate and phosphate containing molecules of the soil organic matter. The second component (mineralisation activity), which explained 18.1 % of the total variance, consisted of carbon (dehydrogenase activity and substrate-induced respiration) and nitrogen (net nitrification and urease activity) mineralisation parameters. Net nitrogen mineralisation

was also included to and discussed in this principal component because of the results of the former PCA of the land use system soils calculated with the aid of the same soil microbial functional parameters and because of another PCA calculated for soil samples of the field experiment providing data for the net nitrogen mineralisation and the remaining functional parameters (data not shown). Both PCA exhibited a close connection between net nitrogen mineralisation and net nitrification and in addition, a highly significant ($P < 0.01$) correlation ($R = 0.677$) between these two nitrogen mineralising parameters was detected. Except the urease activity the parameters of the second component are sensitive correlating oxidising parameters in organic matter degradation being used for estimating effects on soil microbes during xenobiotic presence in order to draw conclusions to microbial biomass (Engelen *et al.*, 1998). In a soil ecosystem possessing a high carbon mineralisation activity simultaneously organic nitrogen compounds being converted to ammonium are released. Hence, the more ammonium is available in the soil the more is transformed to nitrate through nitrification but the fewer is produced by urease activity caused by an urease inhibition due to the enzyme reaction product ammonium explaining the negative algebraic sign of the urease correlation coefficient (Table 21). The third component (metabolic activity) explaining 15.7 % of the total variance was composed of the metabolic quotient and basal respiration, which are both correlating indicators of turn-over processes and instability of the microbial biomass (Wardle *et al.*, 1999). The fourth component (nitrogen transformation potential) explaining 12.7 % of total variance contained N_2O and CO_2 emission rates of the potential denitrification – the only anaerobic process within the soil microbial community functional parameters under study.

3.2.3.5 Discriminant analysis

According to the results obtained from the individual soil microbial function analysis that pesticides if any caused only hardly effects on soil microbial activities the data of the plots treated with pesticides were omitted in this analysis at first. With the aid of a discriminant analysis it was investigated whether it is possible to discriminate between soils of different management systems regarding fertilisation and the degree of land use as conducted for the land use systems (see 3.1.3.6, page 56). For that purpose, soils of the same treatments such as

fallow, reference, conventionally and organically fertilised plots at the same sampling day were expected to be allocated in the respective discriminant group. 94.4 – 99.7 % of the total variance of all group centroids were described by the first (57.8 – 87.9 %) and the second (11.9 – 38.1 %) canonical discriminant functions (CDF) and canonical correlation coefficients close to 1 (CDF 1: 0.942 – 0.990 and CDF 2: 0.910 – 0.942) indicated strong correlations between discriminant scores and groups. Very low Wilks' Lambda values close to 0 (CDF 1: 0.002 – 0.018 and CDF 2: 0.061 – 0.160) showed that the group means were different, which was confirmed by a high significance greater than 95.4 %. The third canonical discriminant functions explaining 0.3 – 5.6 % of the total variance were insignificant. In Figure 19 the first two resulting canonical discriminant functions for all sampling days are plotted and it is obvious that a differentiation among treatments of different fertilisation and the degree of land use was possible. The same kind of discriminant analysis was also applied to the data of the conventionally and organically managed plots whereas it was investigated whether the plots only treated with mineral fertiliser as well as those plus endosulfan, plus chlorothalonil or plus both pesticides and the plots only treated with humus as well as those plus *Bacillus thuringiensis*, plus copper oxychloride or plus both pesticides, respectively, are differentiable. These analyses exhibited that it was neither possible to differentiate between soils treated and non-treated with pesticides nor between soils treated with one or two applications (data not shown). This result was in contrast to Kandeler *et al*. (1996) who could distinguish between soils (Calcaric Phaeozem, Eutric Cambisol and Dystric Lithosol) contaminated with four different heavy metal levels (uncontaminated control, 300 mg kg^{-1} zinc, 100 mg kg^{-1} copper, 50 mg kg^{-1} nickel, 50 mg kg^{-1} vanadium and 3 mg kg^{-1} cadmium as well as two- and threefold concentrations of these heavy metals) by means of a discriminant analysis. As a result, in the present investigation, differences in the activity of soil microbial community functions were only induced by various fertiliser applications confirming the results of the land use system samples where it was possible to distinguish between already established different management systems in the same area whereas effects of pesticide applications could also be excluded due to low pesticide contents in soils of conventional management systems (see 3.1.2.1, page 37).

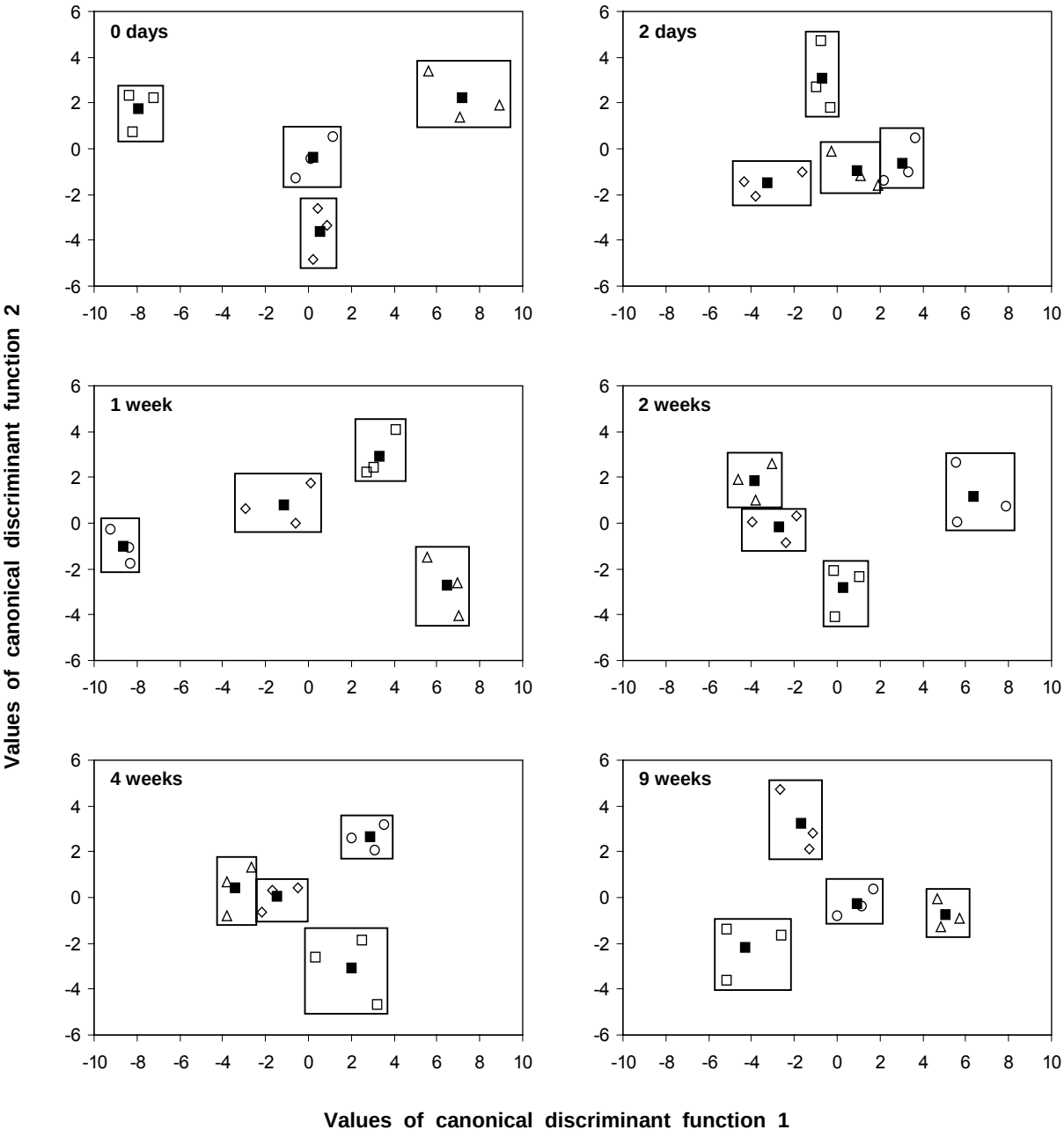

Figure 19: Chronological sequence of the values of the canonical discriminant functions 1 and 2 at all sampling dates after pesticide application; □: fallow, ◇: reference field, △: conventional field, ○: organic field; ■: group centroids.

3.2.3.6 Ecological significance

As already mentioned above, pesticides which were applied at a common agricultural practice level had hardly effects on the investigated soil microbial functions on Mollisols in the surroundings of Buenos Aires. Hence, only the ecological significance of the investigated

parameters between the two management systems with respect to the different fertiliser application and the degree of land use can tendentially be estimated in Figure 20 and 21 whereas Figure 20 exhibits the factor scores of the principal components (PC, Table 21, page 105) and Figure 21 shows those scores after subtraction of the reference means. Parts A, B, C and D exhibit factor scores of PC 1 (Microbial capacity: microbial biomass, acid phosphatase as well as arylsulfatase activity), PC 2 (Mineralisation activity: substrate-induced respiration, dehydrogenase as well as urease activity, net nitrogen mineralisation and net nitrification), PC 3 (Metabolic activity: basal respiration and metabolic quotient) and PC 4 (Nitrogen transformation potential: N_2O and CO_2 emission of the potential denitrification), respectively.

Until one week after pesticide application strong fluctuations of the factor scores but no particular trend with respect to the different treatments were observable. The organically managed plots exhibited the significantly ($P < 0.05$) highest levels in PC 1 two and four weeks after pesticide application indicating the highest microbial capacity when compared to the remaining treatments. This could be explained by the highest contents of total organic carbon (TOC) and total organic nitrogen (TON) in these plots, while the microbial capacity was reduced at the end of the experiment corresponding to the decreasing TOC and TON contents (Figures 17, page 79). The remaining treatments showed similar raising trends of the microbial capacity in the order reference > conventional > fallow plots with the exception of the last sampling day when the latter two were exchanged. The reference soils had a higher microbial activity according to higher TOC and TON contents than the fallows and the conventionally managed plots. Consequently, the microbial capacity was strongly related to TOC and TON contents, which was confirmed by highly significant ($P < 0.01$) correlations ($R = 0.592$ and $R = 0.568$, respectively). Therefore, the organic management system soils were usually better provided with organic matter because of the humus application leading to an enhanced microbial capacity.

In PC 2 the conventionally managed plots had the tendentially highest levels, while the reference and organic treatments showed medium levels during the field experiment and the lowest at the end. The fallow soils had the lowest levels before as well as two and four weeks after pesticide application, while having a medium level at the end. These results could be explained by a highly significant ($P < 0.01$) correlation of the mineralisation activity with

ammonium (R = 0.557) and nitrate (R = 0.509) contents (data not shown). Due to ammonium addition through mineral nitrogen fertilisation to conventionally managed plots net nitrogen mineralisation and particularly net nitrification were heavily activated (Table 19, page 99) leading to the tendentially highest mineralisation activity although the urease activity was inhibited as denoted by a negative correlation coefficient to this component (Table 21, page 105). In a similar manner, the application of easily degradable organic matter in the form of humus induced a temporary increase in carbon mineralisation at first also releasing organic nitrogen compounds and serving as substrate for the nitrogen mineralisation, which then produced ammonium and nitrate. In the soils of the reference only the natural vegetation was incorporated as substrate in contrast to the fallow plots, which were not fertilised at all explaining the lowest levels with the exception at the end of the experiment when the fertilisers of the other treatments were exhausted. Thus, any kind of fertilisation activated partly carbon but strongly nitrogen mineralisation leading to an enhanced mineralisation activity.

By contrast, in PC 3 except two weeks after pesticide application the fallow plots exhibited the significantly ($P < 0.05$) highest levels of metabolic activity throughout the experiment duration, which could be explained by the highest pH value (Figures 17, page 79) correlating highly significantly ($P < 0.01$) with the factor scores of this principal component (R = 0.352). The remaining treatments had similarly low levels whereas those of the reference plots tended to be the lowest. Hence, this indicated that any soil alteration additionally leading to a pH variation animated soil microorganisms being expressed in low levels of the metabolic capacity and high microbial efficiency. This result corresponded to that of the diverse land use systems, in which because of any change of soil management into agricultural land use a strong reduction in metabolic activity was observable indicating an effect caused by soil tillage, which was not performed on the present fallow plots.

Conventional and reference treatments exhibited similar developments in PC 4 with a significant ($P < 0.05$) increase two weeks after pesticide application due to the precipitation event (Figure 16, page 78) increasing the anaerobic process of potential denitrification by more oxygen-free soil properties. Until the end of the experiment all treatments exhibited similar trends whereas the organically managed plots had tendentially the highest and the conventionally managed ones tended to have the lowest levels. This result has already been

explained by higher total organic carbon and nitrogen as well as due to a higher pH value in organic treatments in comparison to the conventional ones (see 3.2.3.3, page 98). Hence, soil oxygen content apparently had a stronger effect on nitrogen transformation potential than soil chemical properties. Nevertheless, a light effect caused by different fertiliser application and thus by reason of diverse management systems was observable.

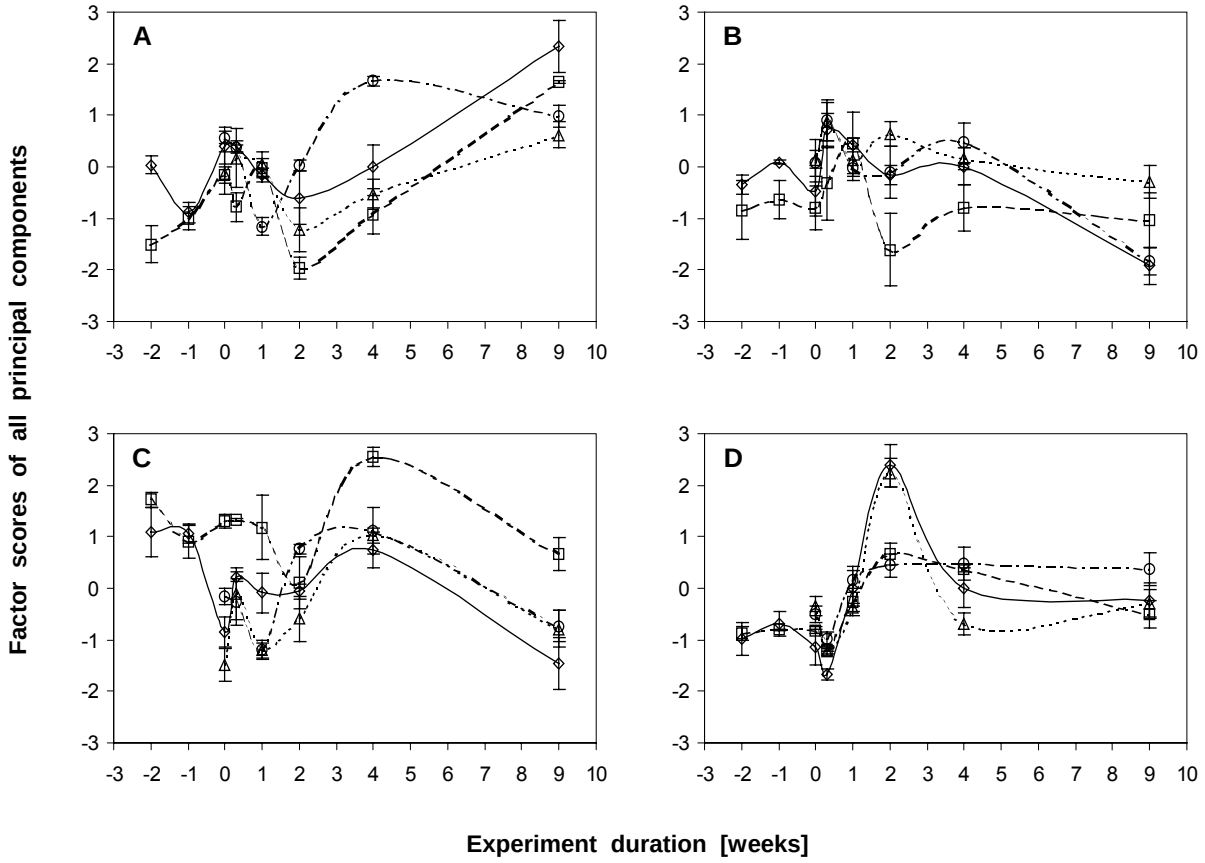

Figure 20: Chronological sequence of the factor scores of all four principal components (PC) of the treatments fallow (□), reference (◇), conventional field (△) and organic field (○) at all sampling dates with standard error bars (N = 3); **A**: PC 1 (Microbial capacity: microbial biomass, acid phosphatase activity and arylsulfatase activity), **B**: PC 2 (Mineralisation activity: substrate-induced respiration, dehydrogenase activity and urease activity, net nitrogen mineralisation and net nitrification), **C**: PC 3 (Metabolic activity: basal respiration and metabolic quotient) and **D**: PC 4 (Nitrogen transformation potential: N_2O and CO_2 emission of the potential denitrification).

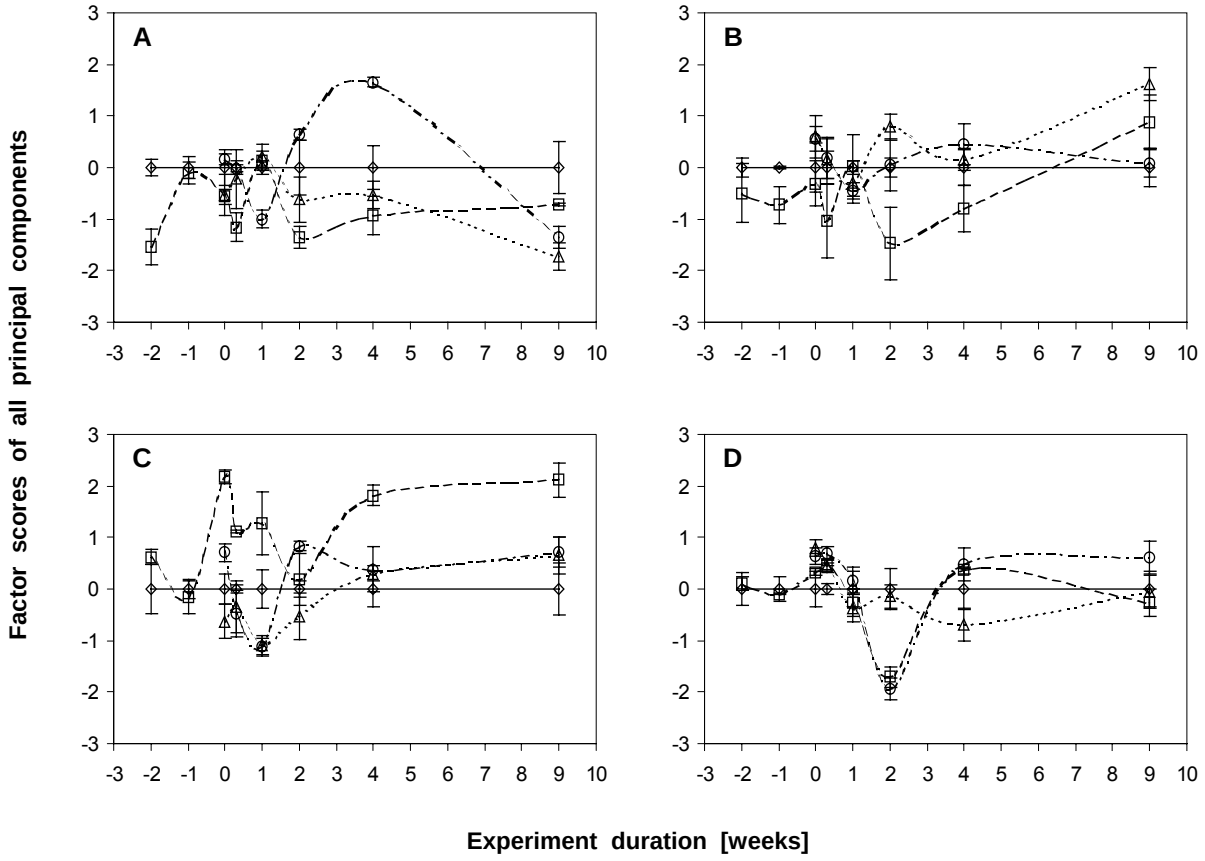

Figure 21: Chronological sequence of the factor scores subtracting those of the reference means of all four principal components (PC) of the treatments fallow (□), reference (◇), conventional field (△) and organic field (○) at all sampling dates with standard error bars (N = 3); **A**: PC 1 (Microbial capacity: microbial biomass, acid phosphatase activity and arylsulfatase activity), **B**: PC 2 (Mineralisation activity: substrate-induced respiration, dehydrogenase activity and urease activity, net nitrogen mineralisation and net nitrification), **C**: PC 3 (Metabolic activity: basal respiration and metabolic quotient) and **D**: PC 4 (Nitrogen transformation potential: N_2O and CO_2 emission of the potential denitrification).

3.2.4 Structural parameters

3.2.4.1 Microbial biomass

The sum of all determined phospholipid fatty acid (PLFA) contents is a measure of viable microbial biomass, since phospholipids are readily degraded after cell death (Zelles, 1997). The organic treatments exhibited significantly ($P < 0.05$) lower microbial biomass contents

compared to the conventional ones at the end of the experiment (Table 22). The temporal fluctuation of soil microbial biomass was higher than differences between diverse treatments such as fertiliser and pesticide applications. Using the soil microbial biomass data computed from substrate-induced respiration (see 3.2.3.2, page 90) a factor of 6.2 ± 1.1 (N = 177) was calculated for the conversion of total PLFA contents (µmol kg^{-1}) into soil microbial biomass carbon (mg kg^{-1}) corresponding to the factor of 5.8 ± 2.3 calculated by Joergensen and Emmerling (2006). Nevertheless, as already mentioned for the PLFA analysis of the land use system soils (see 3.1.4.1, page 60) since conversion factors are only reasonable for particularly defined PLFA, results are further on demonstrated as total PLFA concentrations instead of soil microbial biomass.

The total PLFA content correlated highly significantly ($P < 0.01$) with total organic carbon (TOC) and total organic nitrogen (TON) contents (Figure 22a) indicating a link between nutrient concentrations and the amount of microbial biomass. Furthermore, the total PLFA content exhibited highly significant ($P < 0.01$) correlations to the soil microbial functional parameters such as acid phosphatase, arylsulfatase and dehydrogenase activity, basal respiration, microbial biomass (calculated via substrate-induced respiration) and metabolic quotient (Figure 22b). The correlation to soil microbial biomass was also reported for Typic Agrudalfs (Petersen *et al.*, 1997), for red soils of south-western China (Yao *et al.*, 2000) and for forest soils of northern Germany (Bååth and Anderson, 2003). However, again no correlation to the pH value could be found in contrast to the investigation of Bååth and Anderson (2003).

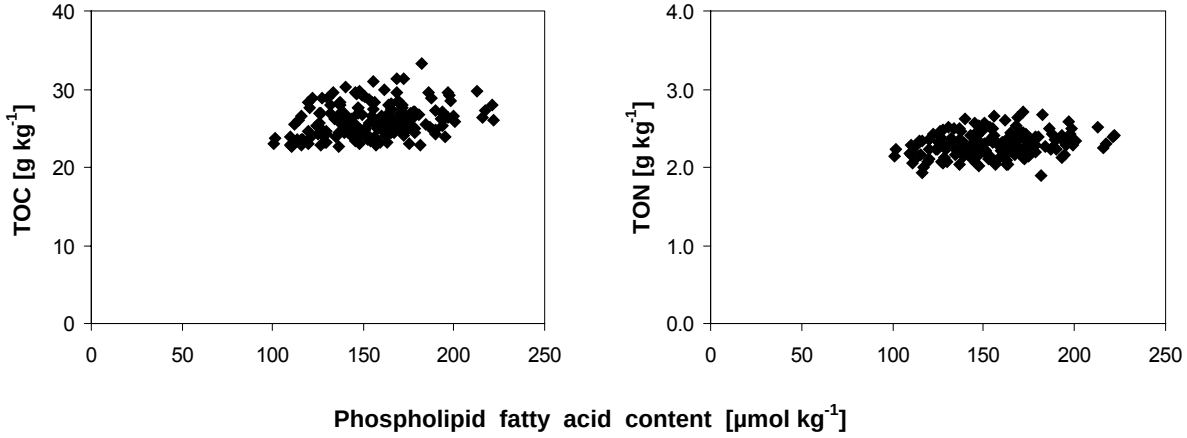

Figure 22a: Correlation plots ($P < 0.01$, N = 177) of total PLFA content with total organic carbon (TOC; R = 0.277) and total organic nitrogen (TON; R = 0.260) contents.

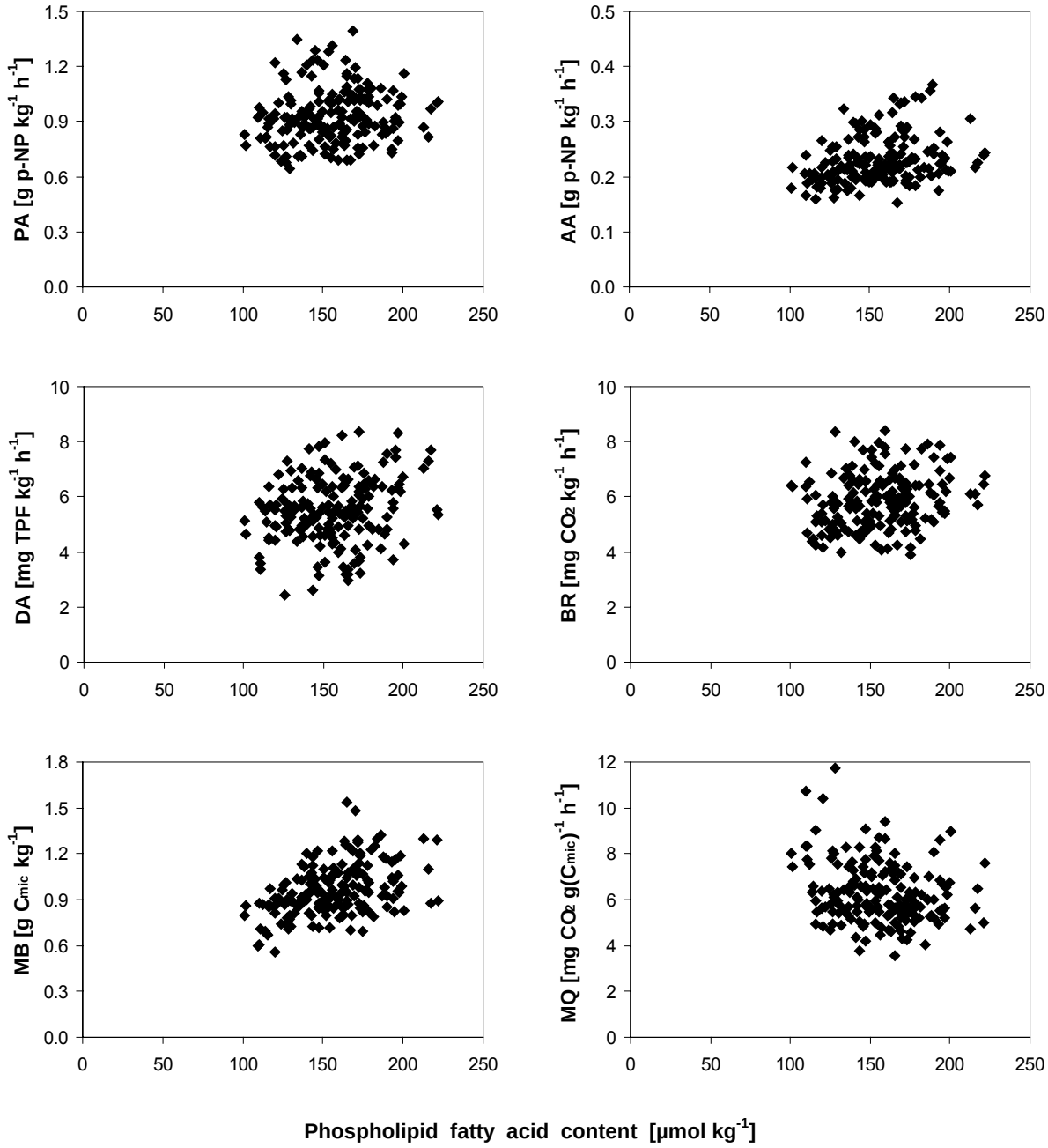

Figure 22b: Correlation plots (P < 0.01, N = 177) of total PLFA content with soil microbial functional parameters such as acid phosphatase activity (PA; R = 0.161), arylsulfatase activity (AA; R = 0.299) dehydrogenase activity (DA; R = 0.189), basal respiration (BR; R = 0.203), microbial biomass (MB; R = 0.440) and metabolic quotient (MQ; R = –0.222).

Table 22: Average total PLFA contents and those subtracting reference means in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Viable microbial biomass (total PLFA) - data [µmol kg^{-1}]								
F	131 ± 11 a AB	118 ± 17 a A	192 ± 20 a D	126 ± 3 a A	161 ± 5 a BCD	117 ± 5 a A	148 ± 10 a ABC	175 ± 8 cd CD
R	146 ± 23 a A	152 ± 7 a A	156 ± 11 a A	194 ± 2 d A	164 ± 14 a A	181 ± 8 e A	167 ± 13 a A	160 ± 8 bcd A
M			154 ± 6 a A	178 ± 10 cd A	143 ± 7 a A	154 ± 9 bcde A	151 ± 8 a A	173 ± 6 cd A
M Es			154 ± 6 a A	183 ± 19 cd A	150 ± 6 a A	145 ± 11 abcd A	174 ± 12 a A	193 ± 15 d A
M Cl			154 ± 6 a A	159 ± 5 bc A	150 ± 6 a A	144 ± 18 abcd A	186 ± 7 a A	173 ± 4 cd A
M Es Cl			154 ± 6 a BCD	133 ± 15 ab AB	134 ± 5 a ABC	126 ± 3 ab A	172 ± 2 a D	156 ± 5 abc CD
H			174 ± 23 a A	160 ± 8 b A	122 ± 5 a A	169 ± 16 cde A	177 ± 4 a A	133 ± 10 a A
H Bt			174 ± 23 a A	144 ± 9 ab A	156 ± 17 a A	176 ± 14 de A	191 ± 12 a A	143 ± 13 ab A
H Cu			174 ± 23 a A	131 ± 4 ab A	141 ± 13 a A	139 ± 20 abc A	163 ± 5 a A	145 ± 4 ab A
H Bt Cu			174 ± 23 a A	162 ± 20 bcd A	135 ± 7 a A	150 ± 5 abcde A	176 ± 9 a A	147 ± 5 ab A
Viable microbial biomass (total PLFA) - data-reference [µmol kg^{-1}]								
F	–15 ± 11 a CDE	–34 ± 17 a BC	36 ± 20 a E	–68 ± 3 a A	–3 ± 5 a CDE	–64 ± 5 a AB	–19 ± 10 a C	16 ± 8 cd DE
R	0 ± 23 a A	0 ± 7 a A	0 ± 11 a A	0 ± 2 d A	0 ± 14 a A	0 ± 8 e A	0 ± 13 a A	0 ± 8 bcd A
M			–1 ± 6 a BC	–16 ± 10 cd AB	–21 ± 7 a AB	–27 ± 9 bcde A	–15 ± 8 a B	14 ± 6 cd C
M Es			–1 ± 6 a ABC	–11 ± 19 cd AB	–14 ± 6 a AB	–37 ± 11 abcd A	7 ± 12 a BC	33 ± 15 d C
M Cl			–1 ± 6 a BC	–35 ± 5 bc A	–14 ± 6 a AB	–37 ± 18 abcd A	20 ± 7 a C	13 ± 4 cd BC
M Es Cl			–1 ± 6 a C	–61 ± 15 ab A	–30 ± 5 a B	–55 ± 3 ab A	5 ± 2 a C	–4 ± 5 abc C
H			18 ± 23 a C	–34 ± 8 b A	–42 ± 5 a A	–12 ± 16 cde ABC	10 ± 4 a BC	–26 ± 10 a AB
H Bt			18 ± 23 a A	–50 ± 9 ab A	–8 ± 17 a A	–6 ± 14 de A	24 ± 12 a A	–17 ± 13 ab A
H Cu			18 ± 23 a C	–63 ± 4 ab A	–23 ± 13 a ABC	–43 ± 20 abc AB	–3 ± 5 a BC	–15 ± 4 ab BC
H Bt Cu			18 ± 23 a A	–32 ± 20 bcd A	–29 ± 7 a A	–31 ± 5 abcde A	10 ± 9 a A	–12 ± 5 ab A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

3.2.4.2 Principal component analysis

Individual PLFA cannot be used to represent specific species, both because an individual bacterial or fungal species can contain numerous fatty acids and because the same fatty acids occur in many different organisms (Zelles, 1999). However, via principle component analysis (PCA) correlating PLFA can be pooled into independent principal components, which then can be allocated to different microbial taxonomic groups according to literature. Moreover, with respect to the investigation of Kelly *et al*. (2003) by means of a PCA of PLFA profiles it was possible to distinguish between soils with different contamination levels of heavy metals by contrasting factor scores resulting from the principal components. In the present PCA, PLFA contents expressed as percentage of total PLFA content were calculated, each PLFA being used as variable. A Kaiser-Meyer-Olkin-measure of sampling adequacy of 0.662 was computed indicating that a PCA was useful for the data, while a value lower than 0.0005 calculated from the Bartlett's Test of Sphericity showed that significant relationships among the variables existed and that the data were suitable for PCA (Backhaus and Erichson, 2003). Resembling the PCA of the land use system samples (see 3.1.4.2, page 63) four principal components, which explained 76.7 % of the total variance were extracted via PCA (Table 23). However, a reasonable classification of the analysed PLFA into specific microbial taxonomic groups regarding this PCA was impossible. The PCA indeed provided high factor loadings for the individual PLFA in the principal components but with different algebraic signs and above all particular PLFA standing for certain microbial groups were allocated into diverse components. This might be explained by very similar soil conditions such as texture, soil density or water content as well as all climatic factors, which might not be effectual for a differentiation. Another reason could be that the experiment duration of nine weeks was too short for a discriminative development of the different taxonomic microbial groups in this agricultural soil under the given treatments regarding fertiliser and pesticide application and thus, no variabilities in PLFA pattern could be detected. On this account, according to the examination of soils of the diverse land use systems the allocation of the microbial taxonomic groups was adopted since similar soil types (Typic Argiudolls) were determined in the same investigation area (Table 11, page 64).

Table 23: Varimax rotated (after Kaiser normalisation) component matrix of the principal component analysis of individual PLFA. Each component combines variables with the highest factor loadings (bold) in a column.

PLFA	Component 1	Component 2	Component 3	Component 4
i15:0	**0.85**	0.21	0.25	0.02
a15:0	**0.83**	-0.21	0.14	0.12
18:2w6,9	**-0.82**	-0.09	0.17	0.03
10Me16:0	**0.62**	0.56	-0.16	-0.05
18:1ω7c	-0.40	**-0.77**	-0.22	-0.02
16:1ω7c	0.01	**-0.76**	-0.17	0.24
i17:0	0.20	**0.77**	0.30	0.27
20:4ω6	-0.25	**0.63**	-0.36	0.27
10Me17:0	-0.33	**0.62**	0.37	0.47
16:1ω5c	0.10	-0.00	**-0.84**	0.12
i16:0	0.55	0.05	**0.77**	0.07
10Me18:0	-0.15	0.31	**0.66**	0.20
a17:0	0.22	0.53	**0.65**	0.13
18:1ω9c	-0.45	-0.05	0.04	**-0.83**
cy17:0	-0.56	0.04	0.13	**0.69**

The temporal variations of PLFA sum contents representing different microbial taxonomic groups are given in Tables 24 to 27. Tables 24 and 26 show mean absolute and relative contents, respectively, and standard errors of all microbial groups in all treatments at every sampling date. However, Tables 25 and 27 exhibit the same absolute and relative contents, respectively, subtracting those of the reference plots at a particular sampling date. The latter allowed a better comparison of treatment effects since subtracting the reference plot PLFA concentrations at individual sampling dates eliminated climatic effects being an important driver for soil microbial properties. Different letters next to the values indicate significant ($P < 0.05$) differences among the data whereas small letters describe differences caused by various treatments at individual sampling dates (vertical), while capital letters describe differences caused by time variations during the field experiment (horizontal).

Table 24a: Average absolute microbial group contents in soils of all treatments during the experiment with standard errors.

Groups	2 weeks before pesticide application	1 week before pesticide application	day of application	2 days after pesticide application	1 week after pesticide application	2 weeks after pesticide application	4 weeks after pesticide application	9 weeks after pesticide application
Treatments								
Gram positive, anaerobic bacteria [µmol kg^{-1}]								
F	8.9 ± 0.5 a ABC	8.3 ± 1.0 a AB	14.0 ± 1.6 a D	8.9 ± 0.1 a ABC	10.7 ± 0.3 a BC	8.2 ± 0.2 a A	11.0 ± 1.0 a C	14.0 ± 0.7 cd D
R	9.9 ± 1.5 a A	10.4 ± 0.4 a AB	12.0 ± 0.9 a ABC	14.6 ± 0.1 d C	12.7 ± 1.2 a BC	14.1 ± 0.8 d C	12.8 ± 1.2 abc BC	13.2 ± 0.6 bc BC
M			11.4 ± 0.3 a A	13.8 ± 0.7 cd BC	11.2 ± 0.6 a A	11.7 ± 0.8 bcd A	12.2 ± 0.7 ab AB	14.3 ± 0.5 cd C
M Es			11.4 ± 0.3 a AB	14.1 ± 1.5 cd BC	11.2 ± 0.3 a AB	10.9 ± 0.8 abc A	13.7 ± 1.1 bcd ABC	15.7 ± 1.2 d C
M Cl			11.4 ± 0.3 a A	12.0 ± 0.4 bcd AB	11.6 ± 0.5 a A	10.4 ± 1.4 ab A	14.8 ± 0.6 cd C	14.0 ± 0.4 cd BC
M Es Cl			11.4 ± 0.3 a BC	10.1 ± 1.2 ab AB	10.2 ± 0.4 a AB	9.2 ± 0.2 ab A	13.8 ± 0.2 bcd D	12.7 ± 0.3 bc CD
H			13.7 ± 1.8 a B	12.1 ± 0.7 bcd AB	9.7 ± 0.3 a A	13.6 ± 1.1 cd B	14.6 ± 0.2 cd B	10.4 ± 0.7 a A
H Bt			13.7 ± 1.8 a A	10.9 ± 0.6 ab A	12.3 ± 1.3 a A	13.9 ± 1.2 d A	15.7 ± 0.9 d A	11.2 ± 1.1 ab A
H Cu			13.7 ± 1.8 a A	9.9 ± 0.5 ab A	10.2 ± 1.8 a A	10.9 ± 1.4 abc A	13.7 ± 0.6 bcd A	11.5 ± 0.4 ab A
H Bt Cu			13.7 ± 1.8 a A	11.9 ± 1.6 bc A	10.6 ± 0.5 a A	11.9 ± 0.3 bcd A	14.7 ± 0.7 cd A	11.6 ± 0.5 ab A
Gram positive, aerobic bacteria [µmol kg^{-1}]								
F	28.1 ± 2.4 a AB	24.6 ± 3.6 a A	43.2 ± 5.7 a D	25.7 ± 0.4 a AB	33.1 ± 1.3 a BC	23.0 ± 0.5 ab A	29.3 ± 2.3 a ABC	36.0 ± 0.9 bc CD
R	31.6 ± 5.5 a A	31.6 ± 0.7 a A	34.8 ± 2.1 a A	43.4 ± 0.8 d A	35.2 ± 3.0 a A	39.3 ± 1.8 d A	35.3 ± 2.6 bc A	32.1 ± 1.3 ab A
M			36.3 ± 2.0 a AB	40.0 ± 1.0 cd B	31.6 ± 1.5 a A	33.0 ± 2.0 bcd A	32.2 ± 1.4 ab A	35.1 ± 1.8 bc AB
M Es			36.3 ± 2.0 a A	40.3 ± 4.0 cd A	31.6 ± 1.5 a A	30.2 ± 2.0 bcd A	36.1 ± 1.4 bc A	39.0 ± 2.7 c A
M Cl			36.3 ± 2.0 a BC	34.6 ± 1.4 bc BC	32.0 ± 1.6 a AB	28.5 ± 3.2 abd A	39.0 ± 1.2 c C	34.4 ± 0.7 b BC
M Es Cl			36.3 ± 2.0 a B	29.8 ± 3.8 ab A	29.3 ± 1.5 a A	26.3 ± 0.8 abd A	36.0 ± 0.9 bc B	31.7 ± 0.8 ab AB
H			35.9 ± 4.7 a BC	36.2 ± 1.4 bc BC	26.0 ± 0.8 a A	38.1 ± 3.7 d C	37.5 ± 1.2 c C	28.0 ± 2.1 a AB
H Bt			35.9 ± 4.7 a A	31.8 ± 1.0 ab A	31.5 ± 3.1 a A	36.9 ± 2.7 cd A	39.1 ± 1.7 c A	28.5 ± 1.8 a A
H Cu			35.9 ± 4.7 a A	28.7 ± 0.5 ab A	28.9 ± 2.3 a A	28.7 ± 3.2 abd A	34.9 ± 1.3 bc A	29.7 ± 0.4 a A
H Bt Cu			35.9 ± 4.7 a A	34.9 ± 4.0 bc A	26.9 ± 1.1 a A	30.6 ± 0.5 bcd A	36.7 ± 2.1 bc A	29.1 ± 0.6 a A
Gram negative bacteria [µmol kg^{-1}]								
F	31.5 ± 4.0 a ABC	29.0 ± 5.6 a A	46.4 ± 4.6 a D	31.1 ± 1.6 a ABC	40.5 ± 0.4 d CD	29.4 ± 2.3 a AB	36.0 ± 4.2 a ABCD	40.4 ± 3.6 bc BCD
R	36.2 ± 6.1 a A	37.1 ± 2.9 a A	35.7 ± 3.6 a A	46.3 ± 1.1 c A	39.6 ± 3.2 cd A	44.2 ± 2.5 a A	40.1 ± 4.3 a A	36.2 ± 2.9 ab A
M			35.4 ± 1.3 a A	40.6 ± 4.2 abc A	32.6 ± 1.9 abc A	36.7 ± 2.5 a A	33.7 ± 2.6 a A	38.7 ± 0.7 bc A
M Es			35.4 ± 1.3 a A	43.3 ± 5.9 bc A	36.5 ± 1.0 bcd A	35.3 ± 3.4 a A	42.1 ± 5.2 a A	44.0 ± 4.3 c A
M Cl			35.4 ± 1.3 a A	37.4 ± 0.8 abc A	36.2 ± 1.7 bcd A	35.9 ± 5.5 a A	44.3 ± 2.2 a A	40.1 ± 1.1 bc A
M Es Cl			35.4 ± 1.3 a B	30.2 ± 3.1 a A	31.6 ± 1.1 ab AB	30.7 ± 0.7 a AB	41.5 ± 0.9 a C	35.4 ± 1.2 ab B
H			41.7 ± 6.2 a A	36.2 ± 2.3 abc A	27.4 ± 0.9 a A	37.0 ± 4.8 a A	40.2 ± 1.8 a A	29.8 ± 2.1 a A
H Bt			41.7 ± 6.2 a A	33.2 ± 3.4 ab A	38.5 ± 4.6 bcd A	41.1 ± 4.4 a A	45.3 ± 3.6 a A	33.4 ± 3.6 ab A
H Cu			41.7 ± 6.2 a A	30.0 ± 2.0 a A	33.9 ± 3.4 abcd A	32.2 ± 5.4 a A	36.3 ± 0.7 a A	32.9 ± 1.3 ab A
H Bt Cu			41.7 ± 6.2 a A	37.7 ± 5.7 abc A	32.7 ± 1.9 abc A	35.2 ± 2.0 a A	40.0 ± 1.6 a A	34.8 ± 1.4 ab A

Data followed by various small letters are significantly (P < 0.05) different indicating treatment effects (vertical) while data followed by various capital letters are significantly (P < 0.05) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 24b: Average absolute microbial group contents in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Fungi [µmol kg^{-1}]								
F	16.6 ± 1.4 a AB	15.1 ± 1.6 a A	22.3 ± 1.8 a C	17.2 ± 0.8 a AB	22.1 ± 1.5 a C	16.2 ± 1.6 a A	20.8 ± 0.9 a BC	23.8 ± 0.9 bcde C
R	19.7 ± 2.8 a A	21.0 ± 1.5 a A	20.5 ± 1.2 a A	24.5 ± 0.2 a A	21.5 ± 1.7 a A	22.8 ± 0.2 a A	21.8 ± 1.8 a A	22.3 ± 1.3 abcd A
M			19.6 ± 1.5 a A	22.8 ± 2.5 a A	18.9 ± 0.9 a A	21.2 ± 0.9 a A	21.0 ± 1.1 a A	24.8 ± 0.7 cde A
M Es			19.6 ± 1.5 a A	23.2 ± 2.2 a A	21.1 ± 1.9 a A	20.1 ± 2.1 a A	25.1 ± 2.3 a A	27.6 ± 2.7 e A
M Cl			19.6 ± 1.5 a A	21.5 ± 0.7 a AB	20.6 ± 0.6 a A	22.6 ± 2.5 a ABC	26.0 ± 1.3 a C	24.9 ± 0.8 de BC
M Es Cl			19.6 ± 1.5 a AB	17.7 ± 2.1 a A	18.4 ± 0.5 a AB	18.2 ± 0.8 a A	24.1 ± 0.9 a C	22.2 ± 1.4 abcd BC
H			25.3 ± 3.6 a A	21.3 ± 0.6 a A	16.7 ± 1.7 a A	20.9 ± 2.4 a A	23.4 ± 0.7 a A	18.1 ± 1.9 a A
H Bt			25.3 ± 3.6 a A	19.6 ± 2.0 a A	21.2 ± 2.8 a A	24.2 ± 1.8 a A	25.2 ± 1.8 a A	20.3 ± 2.6 abc A
H Cu			25.3 ± 3.6 a A	17.2 ± 0.9 a A	19.8 ± 1.8 a A	19.0 ± 3.2 a A	20.9 ± 0.9 a A	19.9 ± 0.8 ab A
H Bt Cu			25.3 ± 3.6 a A	22.3 ± 3.2 a A	19.3 ± 1.1 a A	21.7 ± 1.2 a A	23.2 ± 1.3 a A	20.5 ± 0.8 abcd A
Actinomycetes [µmol kg^{-1}]								
F	5.6 ± 0.1 a ABC	5.1 ± 0.5 a AB	8.2 ± 0.9 a DE	5.5 ± 0.1 a ABC	6.2 ± 0.3 a BC	4.7 ± 0.1 a A	6.8 ± 0.4 a CD	8.4 ± 0.3 b E
R	6.2 ± 0.9 a A	6.7 ± 0.3 a AB	7.5 ± 0.4 a ABC	9.2 ± 0.0 f D	7.8 ± 0.7 a BCD	8.2 ± 0.2 e CD	7.8 ± 0.6 abc BCD	8.2 ± 0.3 b BCD
M			7.2 ± 0.4 a A	8.5 ± 0.3 def BC	6.8 ± 0.4 a A	6.9 ± 0.3 bcd A	7.7 ± 0.3 ab AB	9.6 ± 0.4 c C
M Es			7.2 ± 0.4 a AB	8.9 ± 0.6 ef C	6.9 ± 0.6 a A	6.5 ± 0.4 bc A	8.5 ± 0.2 bcd BC	11.0 ± 0.7 c D
M Cl			7.2 ± 0.4 a AB	7.7 ± 0.6 cde BC	6.9 ± 0.3 a AB	6.1 ± 0.7 abc A	9.2 ± 0.4 d D	9.8 ± 0.3 c CD
M Es Cl			7.2 ± 0.4 a BC	6.3 ± 0.6 abc AB	6.4 ± 0.4 a AB	5.5 ± 0.0 ab A	8.3 ± 0.1 bcd D	8.3 ± 0.3 b CD
H			8.4 ± 1.2 a B	7.3 ± 0.4 bcd AB	5.7 ± 0.4 a A	8.5 ± 0.8 e B	8.5 ± 0.1 bcd B	6.3 ± 0.4 a A
H Bt			8.4 ± 1.2 a AB	6.4 ± 0.3 abc A	6.6 ± 0.6 a A	8.1 ± 0.7 de AB	9.3 ± 0.5 cd B	6.5 ± 0.4 a A
H Cu			8.4 ± 1.2 a A	6.0 ± 0.0 ab A	6.3 ± 0.9 a A	6.3 ± 0.8 bc A	8.4 ± 0.4 bcd A	6.8 ± 0.2 a A
H Bt Cu			8.4 ± 1.2 a A	7.1 ± 0.8 bc A	5.8 ± 0.2 a A	7.1 ± 0.2 cde A	8.6 ± 0.6 bcd A	6.5 ± 0.1 a A
Protozoa [µmol kg^{-1}]								
F	1.6 ± 0.1 a BC	1.4 ± 0.1 a AB	2.1 ± 0.2 a DE	1.3 ± 0.0 bcd AB	1.3 ± 0.1 abc A	1.5 ± 0.0 a AB	1.8 ± 0.1 c CD	2.5 ± 0.0 e E
R	1.7 ± 0.3 a AB	1.5 ± 0.1 a A	1.6 ± 0.1 a A	1.8 ± 0.0 e AB	1.5 ± 0.2 abcd A	2.1 ± 0.0 a BC	1.7 ± 0.1 bc AB	2.3 ± 0.1 cde C
M			1.4 ± 0.1 a AB	1.7 ± 0.1 de B	1.2 ± 0.0 ab A	1.7 ± 0.1 a B	1.3 ± 0.1 a A	2.1 ± 0.1 cde C
M Es			1.4 ± 0.1 a AB	1.7 ± 0.2 de B	1.3 ± 0.1 ab A	1.6 ± 0.1 a AB	1.4 ± 0.1 ab AB	2.4 ± 0.1 de C
M Cl			1.4 ± 0.1 a A	1.5 ± 0.1 cde A	1.3 ± 0.1 abc A	1.6 ± 0.2 a A	1.6 ± 0.1 abc A	2.1 ± 0.0 cde B
M Es Cl			1.4 ± 0.1 a B	1.3 ± 0.1 bc AB	1.2 ± 0.0 a A	1.4 ± 0.1 a B	1.4 ± 0.0 ab AB	2.0 ± 0.1 cd C
H			1.7 ± 0.3 a BC	1.0 ± 0.1 ab A	1.6 ± 0.1 bcd B	2.1 ± 0.1 a C	2.7 ± 0.1 d D	1.4 ± 0.2 ab AB
H Bt			1.7 ± 0.3 a BC	0.9 ± 0.0 a A	1.7 ± 0.2 d BC	2.0 ± 0.2 a C	2.8 ± 0.1 d D	1.5 ± 0.1 ab B
H Cu			1.7 ± 0.3 a BC	1.2 ± 0.2 abc A	1.5 ± 0.2 abcd AB	2.3 ± 0.7 a BC	2.6 ± 0.1 d C	1.6 ± 0.0 ab AB
H Bt Cu			1.7 ± 0.3 a B	1.2 ± 0.1 abc A	1.6 ± 0.1 cd B	1.8 ± 0.0 a B	2.9 ± 0.2 d C	1.9 ± 0.4 bc B

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 25a: Average absolute microbial group contents subtracting reference means in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Gram positive, anaerobic bacteria [µmol kg^{-1}]								
F	–0.9 ± 0.5 a BCD	–2.1 ± 1.0 a B	2.0 ± 1.6 a D	–5.7 ± 0.1 a A	–2.0 ± 0.3 a B	–5.9 ± 0.2 a A	–1.8 ± 1.0 a B	0.8 ± 0.7 cd CD
R	0.0 ± 1.5 a A	0.0 ± 0.4 a A	0.0 ± 0.9 a A	0.0 ± 0.1 d A	0.0 ± 1.2 a A	0.0 ± 0.8 d A	0.0 ± 1.2 abc A	0.0 ± 0.6 bc A
M			–0.6 ± 0.3 a AB	–0.8 ± 0.7 cd AB	–1.5 ± 0.6 a A	–2.4 ± 0.8 bcd A	–0.6 ± 0.7 ab AB	1.1 ± 0.5 cd B
M Es			–0.6 ± 0.3 a AB	–0.5 ± 1.5 cd ABC	–1.5 ± 0.3 a AB	–3.2 ± 0.8 abc A	0.8 ± 1.1 bcd BC	2.5 ± 1.2 d C
M Cl			–0.6 ± 0.3 a BC	–2.6 ± 0.4 bcd AB	–1.2 ± 0.5 a BC	–3.7 ± 1.4 ab A	2.0 ± 0.6 cd D	0.8 ± 0.4 cd CD
M Es Cl			–0.6 ± 0.3 a C	–4.5 ± 1.2 ab A	–2.5 ± 0.4 a B	–4.8 ± 0.2 ab A	1.0 ± 0.2 bcd C	–0.5 ± 0.3 bc C
H			1.7 ± 1.8 a B	–2.5 ± 0.7 bcd A	–3.1 ± 0.3 a A	–0.5 ± 1.1 cd AB	1.7 ± 0.2 cd B	–2.8 ± 0.7 a A
H Bt			1.7 ± 1.8 a BC	–3.7 ± 0.6 ab A	–0.4 ± 1.3 a ABC	–0.2 ± 1.2 d ABC	2.9 ± 0.9 d C	–2.0 ± 1.1 ab AB
H Cu			1.7 ± 1.8 a C	–4.7 ± 0.5 ab A	–2.5 ± 1.8 a AB	–3.2 ± 1.4 abc A	0.8 ± 0.6 bcd BC	–1.7 ± 0.4 ab ABC
H Bt Cu			1.7 ± 1.8 a BC	–2.7 ± 1.6 bc A	–2.2 ± 0.5 a A	–2.2 ± 0.3 bcd A	1.8 ± 0.7 cd C	–1.6 ± 0.5 ab AB
Gram positive, aerobic bacteria [µmol kg^{-1}]								
F	–3.6 ± 2.4 a BCD	–7.0 ± 3.6 a B	8.4 ± 5.7 a D	–17.7 ± 0.4 a A	–2.1 ± 1.3 a BCD	–16.3 ± 0.5 ab A	–6.0 ± 2.3 a B	3.9 ± 0.9 bc CD
R	0.0 ± 5.5 a A	0.0 ± 0.7 a A	0.0 ± 2.1 a A	0.0 ± 0.8 d A	0.0 ± 3.0 a A	0.0 ± 1.8 d A	0.0 ± 2.6 bc A	0.0 ± 1.3 ab A
M			1.6 ± 2.0 a BC	–3.4 ± 1.0 cd AB	–3.6 ± 1.5 a A	–6.4 ± 2.0 bcd A	–3.2 ± 1.4 ab AB	2.9 ± 1.8 bc C
M Es			1.6 ± 2.0 a BC	–3.1 ± 4.0 cd AB	–3.6 ± 1.5 a AB	–9.1 ± 2.0 bcd A	0.8 ± 1.4 bc BC	6.8 ± 2.7 c C
M Cl			1.6 ± 2.0 a CD	–8.8 ± 1.4 bc AB	–3.2 ± 1.6 a BC	–10.8 ± 3.2 abd A	3.6 ± 1.2 c D	2.2 ± 0.7 b CD
M Es Cl			1.6 ± 2.0 a C	–13.6 ± 3.8 ab A	–6.0 ± 1.5 a B	–13.1 ± 0.8 abd A	0.7 ± 0.9 bc C	–0.4 ± 0.8 ab BC
H			1.1 ± 4.7 a A	–7.2 ± 1.4 bc A	–9.2 ± 0.8 a A	–1.2 ± 3.7 d A	2.2 ± 1.2 c A	–4.1 ± 2.1 a A
H Bt			1.1 ± 4.7 a B	–11.6 ± 1.0 ab A	–3.8 ± 3.1 a AB	–2.4 ± 2.7 cd B	3.7 ± 1.7 c B	–3.7 ± 1.8 a AB
H Cu			1.1 ± 4.7 a C	–14.7 ± 0.5 ab A	–6.4 ± 2.3 a BC	–10.7 ± 3.2 abd AB	–0.4 ± 1.3 bc C	–2.4 ± 0.4 a C
H Bt Cu			1.1 ± 4.7 a B	–8.5 ± 4.0 bc A	–8.4 ± 1.1 a A	–8.7 ± 0.5 bcd A	1.3 ± 2.1 bc B	–3.0 ± 0.6 a AB
Gram negative bacteria [µmol kg^{-1}]								
F	–4.7 ± 4.0 a ABCD	–8.1 ± 5.6 a ABC	10.6 ± 4.6 a E	–15.2 ± 1.6 a A	0.9 ± 0.4 d CDE	–14.9 ± 2.3 a AB	–4.1 ± 4.2 a BCD	4.2 ± 3.6 bc DE
R	0.0 ± 6.1 a A	0.0 ± 2.9 a A	0.0 ± 3.6 a A	0.0 ± 1.1 c A	0.0 ± 3.2 cd A	0.0 ± 2.5 a A	0.0 ± 4.3 a A	0.0 ± 2.9 ab A
M			–0.3 ± 1.3 a A	–5.6 ± 4.2 abc A	–7.0 ± 1.9 abc A	–7.6 ± 2.5 a A	–6.4 ± 2.6 a A	2.5 ± 0.7 bc A
M Es			–0.3 ± 1.3 a A	–2.9 ± 5.9 bc A	–3.1 ± 1.0 bcd A	–9.0 ± 3.4 a A	2.0 ± 5.2 a A	7.9 ± 4.3 c A
M Cl			–0.3 ± 1.3 a BC	–8.9 ± 0.8 abc A	–3.4 ± 1.7 bcd ABC	–8.3 ± 5.5 a AB	4.1 ± 2.2 a C	3.9 ± 1.1 bc C
M Es Cl			–0.3 ± 1.3 a C	–16.1 ± 3.1 a A	–8.0 ± 1.1 ab B	–13.6 ± 0.7 a A	1.4 ± 0.9 a C	–0.8 ± 1.2 ab C
H			5.9 ± 6.2 a C	–10.1 ± 2.3 abc AB	–12.2 ± 0.9 a A	–7.2 ± 4.8 a AB	0.1 ± 1.8 a BC	–6.4 ± 2.1 a AB
H Bt			5.9 ± 6.2 a A	–13.1 ± 3.4 ab A	–1.1 ± 4.6 bcd A	–3.1 ± 4.4 a A	5.2 ± 3.6 a A	–2.8 ± 3.6 ab A
H Cu			5.9 ± 6.2 a C	–16.3 ± 2.0 a A	–5.7 ± 3.4 abcd AB	–12.1 ± 5.4 a AB	–3.8 ± 0.7 a BC	–3.3 ± 1.3 ab BC
H Bt Cu			5.9 ± 6.2 a A	–8.6 ± 5.7 abc A	–6.9 ± 1.9 abc A	–9.0 ± 2.0 a A	–0.1 ± 1.6 a A	–1.4 ± 1.4 ab A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 25b: Average absolute microbial group contents subtracting reference means in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Fungi [µmol kg^{-1}]								
F	–3.0 ± 1.4 a BC	–5.9 ± 1.6 a AB	1.8 ± 1.8 a D	–7.4 ± 0.8 a A	0.6 ± 1.5 a CD	–6.7 ± 1.6 a AB	–0.9 ± 0.9 a CD	1.5 ± 0.9 bcde D
R	0.0 ± 2.8 a A	0.0 ± 1.5 a A	0.0 ± 1.2 a A	0.0 ± 0.2 a A	0.0 ± 1.7 a A	0.0 ± 0.2 a A	0.0 ± 1.8 a A	0.0 ± 1.3 abcd A
M			–0.9 ± 1.5 a A	–1.7 ± 2.5 a A	–2.7 ± 0.9 a A	–1.6 ± 0.9 a A	–0.7 ± 1.1 a A	2.5 ± 0.7 cde A
M Es			–0.9 ± 1.5 a A	–1.3 ± 2.2 a A	–0.4 ± 1.9 a A	–2.8 ± 2.1 a A	3.3 ± 2.3 a A	5.3 ± 2.7 e A
M Cl			–0.9 ± 1.5 a AB	–3.0 ± 0.7 a A	–0.9 ± 0.6 a AB	–0.3 ± 2.5 a AB	4.3 ± 1.3 a C	2.7 ± 0.8 de BC
M Es Cl			–0.9 ± 1.5 a BCD	–6.9 ± 2.1 a A	–3.1 ± 0.5 a ABC	–4.7 ± 0.8 a AB	2.4 ± 0.9 a D	0.0 ± 1.4 abcd CD
H			4.8 ± 3.6 a C	–3.2 ± 0.6 a AB	–4.8 ± 1.7 a A	–1.9 ± 2.4 a AB	1.6 ± 0.7 a BC	–4.2 ± 1.9 a AB
H Bt			4.8 ± 3.6 a A	–4.9 ± 2.0 a A	–0.3 ± 2.8 a A	1.4 ± 1.8 a A	3.4 ± 1.8 a A	–2.0 ± 2.6 abc A
H Cu			4.8 ± 3.6 a B	–7.3 ± 0.9 a A	–1.7 ± 1.8 a AB	–3.9 ± 3.2 a A	–0.8 ± 0.9 a AB	–2.4 ± 0.8 ab A
H Bt Cu			4.8 ± 3.6 a A	–2.2 ± 3.2 a A	–2.2 ± 1.1 a A	–1.1 ± 1.2 a A	1.5 ± 1.3 a A	–1.7 ± 0.8 abcd A
Actinomycetes [µmol kg^{-1}]								
F	–0.6 ± 0.1 a BCD	–1.5 ± 0.5 a B	0.7 ± 0.9 a D	–3.7 ± 0.1 a A	–1.6 ± 0.3 a B	–3.6 ± 0.1 a A	–1.0 ± 0.4 a BC	0.2 ± 0.3 b CD
R	0.0 ± 0.9 a A	0.0 ± 0.3 a A	0.0 ± 0.4 a A	0.0 ± 0.0 f A	0.0 ± 0.7 a A	0.0 ± 0.2 e A	0.0 ± 0.6 abc A	0.0 ± 0.3 b A
M			–0.3 ± 0.4 a AB	–0.7 ± 0.3 def AB	–1.0 ± 0.4 a AB	–1.4 ± 0.3 bcd A	–0.1 ± 0.3 ab B	1.4 ± 0.4 c C
M Es			–0.3 ± 0.4 a AB	–0.3 ± 0.6 ef AB	–0.9 ± 0.6 a A	–1.7 ± 0.4 bc A	0.7 ± 0.2 bcd B	2.9 ± 0.7 c C
M Cl			–0.3 ± 0.4 a B	–1.5 ± 0.6 cde AB	–0.9 ± 0.3 a AB	–2.1 ± 0.7 abc A	1.4 ± 0.4 d C	1.7 ± 0.3 c C
M Es Cl			–0.3 ± 0.4 a C	–2.9 ± 0.6 abc A	–1.4 ± 0.4 a B	–2.8 ± 0.0 ab A	0.5 ± 0.1 bcd C	0.1 ± 0.3 b C
H			0.9 ± 1.2 a B	–1.9 ± 0.4 bcd A	–2.1 ± 0.4 a A	0.2 ± 0.8 e B	0.7 ± 0.1 bcd B	–1.9 ± 0.4 a A
H Bt			0.9 ± 1.2 a C	–2.8 ± 0.3 abc A	–1.2 ± 0.6 a AB	–0.1 ± 0.7 de BC	1.5 ± 0.5 cd C	–1.7 ± 0.4 a AB
H Cu			0.9 ± 1.2 a C	–3.2 ± 0.0 ab A	–1.5 ± 0.9 a AB	–1.9 ± 0.8 bc A	0.6 ± 0.4 bcd BC	–1.4 ± 0.2 a AB
H Bt Cu			0.9 ± 1.2 a B	–2.1 ± 0.8 bc A	–2.0 ± 0.2 a A	–1.2 ± 0.2 cde A	0.9 ± 0.6 bcd B	–1.7 ± 0.1 a A
Protozoa [µmol kg^{-1}]								
F	–0.2 ± 0.1 a BC	–0.1 ± 0.1 a C	0.5 ± 0.2 a E	–0.5 ± 0.0 bcd AB	–0.1 ± 0.1 abc C	0.6 ± 0.0 a A	0.1 ± 0.1 c CD	0.2 ± 0.0 e DE
R	0.0 ± 0.3 a A	0.0 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.0 e A	0.0 ± 0.2 abcd A	0.0 ± 0.0 a A	0.0 ± 0.1 bc A	0.0 ± 0.1 cde A
M			–0.2 ± 0.1 a A	–0.1 ± 0.1 de A	–0.2 ± 0.0 ab A	–0.4 ± 0.1 a A	–0.4 ± 0.1 a A	–0.2 ± 0.1 cde A
M Es			–0.2 ± 0.1 a BC	–0.1 ± 0.2 de BC	–0.2 ± 0.1 ab ABC	–0.5 ± 0.1 a A	–0.3 ± 0.1 ab AB	0.1 ± 0.1 de C
M Cl			–0.2 ± 0.1 a A	–0.3 ± 0.1 cde A	–0.2 ± 0.1 abc A	–0.5 ± 0.2 a A	–0.1 ± 0.1 abc A	–0.2 ± 0.0 cde A
M Es Cl			–0.2 ± 0.1 a C	–0.5 ± 0.1 bc AB	–0.3 ± 0.0 a BC	–0.6 ± 0.1 a A	–0.3 ± 0.0 ab BC	–0.2 ± 0.1 cd AB
H			0.1 ± 0.3 a B	–0.8 ± 0.1 ab A	0.1 ± 0.1 bcd B	0.1 ± 0.1 a B	1.0 ± 0.1 d C	–0.9 ± 0.2 ab A
H Bt			0.1 ± 0.3 a B	–0.9 ± 0.0 a A	0.3 ± 0.2 d B	–0.0 ± 0.2 a B	1.1 ± 0.1 d C	–0.8 ± 0.1 ab A
H Cu			0.1 ± 0.3 a AB	–0.6 ± 0.2 abc A	0.0 ± 0.2 abcd AB	0.2 ± 0.7 a AB	0.9 ± 0.1 d B	–0.7 ± 0.0 ab A
H Bt Cu			0.1 ± 0.3 a B	–0.6 ± 0.1 abc A	0.2 ± 0.1 cd B	–0.3 ± 0.0 a AB	1.2 ± 0.2 d C	–0.4 ± 0.4 bc AB

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 26a: Average relative microbial group abundances in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Gram positive, anaerobic bacteria [%]								
F	6.9 ± 0.2 a AB	7.1 ± 0.1 a BC	7.3 ± 0.1 a C	7.1 ± 0.2 a BC	6.7 ± 0.1 a A	7.0 ± 0.1 a ABC	7.4 ± 0.2 a C	8.0 ± 0.1 ab D
R	6.7 ± 0.1 a A	6.9 ± 0.0 a A	7.7 ± 0.0 b B	7.5 ± 0.0 bc B	7.7 ± 0.1 bc B	7.8 ± 0.1 cd B	7.7 ± 0.1 ab B	8.3 ± 0.0 c C
M			7.4 ± 0.1 a A	7.8 ± 0.1 c B	7.8 ± 0.1 c B	7.6 ± 0.1 c A	8.1 ± 0.0 cde C	8.2 ± 0.0 c C
M Es			7.4 ± 0.1 a A	7.7 ± 0.1 c BC	7.5 ± 0.1 bc AB	7.5 ± 0.1 bc AB	7.8 ± 0.1 bc C	8.1 ± 0.0 bc D
M Cl			7.4 ± 0.1 a B	7.6 ± 0.1 bc BC	7.7 ± 0.0 bc C	7.2 ± 0.1 b A	8.0 ± 0.0 bcd D	8.1 ± 0.1 bc D
M Es Cl			7.4 ± 0.1 a AB	7.6 ± 0.1 bc AB	7.6 ± 0.0 bc B	7.3 ± 0.0 b A	8.1 ± 0.2 cde C	8.1 ± 0.1 bc C
H			7.9 ± 0.1 b B	7.6 ± 0.1 bc A	7.9 ± 0.1 c B	8.1 ± 0.1 e BC	8.2 ± 0.1 de C	7.8 ± 0.1 a AB
H Bt			7.9 ± 0.1 b B	7.6 ± 0.1 bc A	7.9 ± 0.1 c B	7.9 ± 0.1 de B	8.3 ± 0.0 de C	7.9 ± 0.0 a B
H Cu			7.9 ± 0.1 b A	7.6 ± 0.2 bc A	7.1 ± 0.7 b A	7.9 ± 0.1 de A	8.4 ± 0.1 e B	8.0 ± 0.0 ab AB
H Bt Cu			7.9 ± 0.1 b B	7.3 ± 0.1 ab A	7.8 ± 0.1 c B	7.9 ± 0.1 de B	8.3 ± 0.0 e C	7.9 ± 0.1 a B
Gram positive, aerobic bacteria [%]								
F	21.5 ± 0.0 a BC	20.9 ± 0.1 a AB	22.4 ± 0.7 a C	20.5 ± 0.3 a AB	20.5 ± 0.3 abc AB	19.7 ± 0.4 a A	19.8 ± 0.3 a A	20.6 ± 0.4 a AB
R	21.5 ± 0.7 a BC	20.9 ± 0.6 a AB	22.4 ± 0.2 a C	22.4 ± 0.3 a C	21.5 ± 0.2 cd BC	21.7 ± 0.1 de BC	21.2 ± 0.5 a ABC	20.2 ± 0.5 a A
M			23.5 ± 1.0 a C	22.6 ± 1.0 a BC	22.1 ± 0.1 d ABC	21.3 ± 0.4 cd AB	21.3 ± 0.4 a AB	20.2 ± 0.3 a A
M Es			23.5 ± 1.0 a C	22.1 ± 0.4 a BC	21.0 ± 0.4 bc AB	21.0 ± 0.2 bcd AB	20.9 ± 0.7 a AB	20.2 ± 0.2 a A
M Cl			23.5 ± 1.0 a C	21.8 ± 0.3 a B	21.3 ± 0.4 cd AB	19.8 ± 0.3 ab A	20.9 ± 0.3 a AB	19.9 ± 0.1 a A
M Es Cl			23.5 ± 1.0 a C	22.3 ± 0.3 a BC	21.8 ± 0.5 d AB	20.8 ± 0.3 abcd AB	21.0 ± 0.4 a AB	20.3 ± 0.2 a A
H			20.6 ± 0.3 a A	22.7 ± 0.3 a B	21.3 ± 0.2 cd A	22.5 ± 0.0 e B	21.2 ± 0.4 a A	21.1 ± 0.0 a A
H Bt			20.6 ± 0.3 a A	22.1 ± 0.8 a A	20.2 ± 0.6 ab A	21.1 ± 0.5 c A	20.5 ± 0.4 a A	20.0 ± 0.5 a A
H Cu			20.6 ± 0.3 a A	22.0 ± 0.9 a A	20.5 ± 0.3 abc A	20.8 ± 0.6 bcd A	21.4 ± 0.2 a A	20.6 ± 0.3 a A
H Bt Cu			20.6 ± 0.3 a AB	21.6 ± 0.25 a B	19.9 ± 0.3 a A	20.4 ± 0.5 abcd A	20.8 ± 0.3 a AB	19.8 ± 0.4 a A
Gram negative bacteria [%]								
F	23.9 ± 1.1 a A	24.3 ± 1.4 a A	24.2 ± 0.7 a A	24.7 ± 0.8 a A	25.2 ± 1.0 a A	25.0 ± 1.4 a A	24.2 ± 1.5 a A	23.0 ± 1.1 a A
R	24.7 ± 0.5 a A	24.4 ± 0.8 a A	22.9 ± 0.8 a A	23.9 ± 0.5 a A	24.2 ± 0.6 a A	24.4 ± 0.4 a A	24.0 ± 1.0 a A	22.6 ± 0.7 a A
M			23.0 ± 0.7 a A	22.8 ± 1.2 a A	22.8 ± 0.3 a A	23.7 ± 0.7 a A	22.2 ± 0.6 a A	22.3 ± 0.6 a A
M Es			23.0 ± 0.7 a A	23.6 ± 0.9 a A	24.3 ± 0.9 a A	24.3 ± 0.5 a A	24.0 ± 1.2 a A	22.8 ± 0.5 a A
M Cl			23.0 ± 0.7 a A	23.6 ± 0.5 a A	24.1 ± 0.6 a A	24.8 ± 0.8 a A	23.8 ± 0.6 a A	23.2 ± 0.1 a A
M Es Cl			23.0 ± 0.7 a A	22.7 ± 0.2 a A	23.6 ± 0.5 a A	24.3 ± 0.4 a A	24.1 ± 0.7 a A	22.7 ± 0.5 a A
H			23.9 ± 0.3 a A	22.6 ± 0.4 a A	22.4 ± 0.3 a A	21.7 ± 0.8 a A	22.7 ± 0.8 a A	22.4 ± 0.6 a A
H Bt			23.9 ± 0.3 a A	22.9 ± 0.9 a A	24.6 ± 1.0 a A	23.3 ± 1.1 a A	23.8 ± 0.4 a A	23.3 ± 0.3 a A
H Cu			23.9 ± 0.3 a A	22.8 ± 0.9 a A	23.9 ± 0.3 a A	23.0 ± 0.6 a A	22.3 ± 0.3 a A	22.8 ± 0.3 a A
H Bt Cu			23.9 ± 0.3 a A	23.2 ± 0.6 a A	24.2 ± 0.5 a A	23.4 ± 0.6 a A	22.8 ± 0.3 a A	23.7 ± 0.3 a A

Data followed by various small letters are significantly (P < 0.05) different indicating treatment effects (vertical) while data followed by various capital letters are significantly (P < 0.05) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 26b: Average relative microbial group abundances in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Fungi [%]								
F	12.7 ± 0.3 a A	12.9 ± 0.4 a A	11.7 ± 0.5 a A	13.7 ± 0.4 a A	13.7 ± 0.5 a A	13.7 ± 0.9 bc A	14.2 ± 1.2 a A	13.6 ± 0.1 a A
R	13.5 ± 0.4 a A	13.8 ± 0.3 a A	13.2 ± 0.3 bc A	12.7 ± 0.0 a A	13.1 ± 0.2 a A	12.6 ± 0.5 ab A	13.0 ± 0.2 a A	13.9 ± 0.4 a A
M			12.7 ± 0.7 ab A	12.8 ± 0.8 a A	13.2 ± 0.3 a A	13.8 ± 0.2 bc A	13.9 ± 0.2 a A	14.3 ± 0.1 a A
M Es			12.7 ± 0.7 ab A	12.8 ± 0.5 a A	14.0 ± 0.7 a A	13.8 ± 0.4 bc A	14.4 ± 0.3 a A	14.2 ± 0.3 a A
M Cl			12.7 ± 0.7 ab A	13.5 ± 0.1 a AB	13.7 ± 0.2 a AB	15.7 ± 0.4 d C	14.0 ± 0.3 a B	14.4 ± 0.3 a B
M Es Cl			12.7 ± 0.7 ab A	13.2 ± 0.1 a A	13.8 ± 0.1 a A	14.4 ± 0.3 cd A	14.0 ± 0.4 a A	14.2 ± 0.4 a A
H			14.5 ± 0.2 c A	13.4 ± 0.5 a A	13.6 ± 0.8 a A	12.3 ± 0.5 a A	13.2 ± 0.2 a A	13.5 ± 0.3 a A
H Bt			14.5 ± 0.2 c A	13.5 ± 0.6 a A	13.5 ± 0.5 a A	13.8 ± 0.4 bc A	13.2 ± 0.1 a A	14.1 ± 0.5 a A
H Cu			14.5 ± 0.2 c D	13.1 ± 0.3 a AB	14.0 ± 0.3 a CD	13.6 ± 0.3 abc ABC	12.8 ± 0.2 a A	13.7 ± 0.2 a BCD
H Bt Cu			14.5 ± 0.2 c A	13.7 ± 0.3 a A	14.2 ± 0.3 a A	14.4 ± 0.4 cd A	13.2 ± 0.3 a A	13.9 ± 0.4 a A
Actinomycetes [%]								
F	4.3 ± 0.3 a BC	4.4 ± 0.2 a BCD	4.2 ± 0.0 a BC	4.4 ± 0.0 a BC	3.8 ± 0.1 a A	4.0 ± 0.1 a AB	4.6 ± 0.0 a CD	4.8 ± 0.1 bc D
R	4.3 ± 0.1 a A	4.4 ± 0.1 a AB	4.8 ± 0.2 a BC	4.7 ± 0.1 a BC	4.7 ± 0.2 c BC	4.6 ± 0.1 bcd AB	4.7 ± 0.3 a ABC	5.1 ± 0.1 cd C
M			4.7 ± 0.1 a A	4.8 ± 0.1 a AB	4.8 ± 0.0 c AB	4.5 ± 0.1 bcd A	5.1 ± 0.2 a B	5.5 ± 0.1 ef C
M Es			4.7 ± 0.1 a A	4.9 ± 0.3 a A	4.5 ± 0.2 bc A	4.5 ± 0.1 bcd A	4.9 ± 0.3 a A	5.7 ± 0.2 f B
M Cl			4.7 ± 0.1 a AB	4.8 ± 0.2 a B	4.6 ± 0.1 bc AB	4.3 ± 0.1 ab A	4.9 ± 0.1 a B	5.7 ± 0.1 f C
M Es Cl			4.7 ± 0.1 a B	4.8 ± 0.1 a B	4.7 ± 0.1 c B	4.3 ± 0.1 bc A	4.8 ± 0.1 a B	5.3 ± 0.0 de C
H			4.8 ± 0.1 a A	4.5 ± 0.1 a A	4.6 ± 0.2 bc A	5.0 ± 0.0 e A	4.8 ± 0.0 a A	4.7 ± 0.1 ab A
H Bt			4.8 ± 0.1 a A	4.5 ± 0.2 a A	4.2 ± 0.1 ab A	4.6 ± 0.2 cd A	4.9 ± 0.1 a A	4.6 ± 0.2 ab A
H Cu			4.8 ± 0.1 a A	4.6 ± 0.1 a A	4.4 ± 0.3 bc A	4.6 ± 0.1 bcd A	5.1 ± 0.1 a A	4.7 ± 0.1 ab A
H Bt Cu			4.8 ± 0.1 a C	4.4 ± 0.1 a AB	4.3 ± 0.1 abc A	4.7 ± 0.1 de BC	4.9 ± 0.2 a C	4.4 ± 0.1 a AB
Protozoa [%]								
F	1.2 ± 0.0 a B	1.2 ± 0.1 a B	1.1 ± 0.1 a B	1.1 ± 0.0 c B	0.8 ± 0.0 a A	1.3 ± 0.1 bc BC	1.2 ± 0.0 c BC	1.4 ± 0.0 b C
R	1.2 ± 0.0 a B	1.0 ± 0.1 a AB	1.0 ± 0.0 a AB	0.9 ± 0.0 b AB	0.9 ± 0.0 a A	1.1 ± 0.0 ab B	1.0 ± 0.0 b AB	1.4 ± 0.1 b C
M			0.9 ± 0.1 a A	0.9 ± 0.0 bc A	0.9 ± 0.0 a A	1.1 ± 0.0 a B	0.9 ± 0.0 a A	1.2 ± 0.0 ab C
M Es			0.9 ± 0.1 a A	0.9 ± 0.0 b A	0.8 ± 0.0 a A	1.1 ± 0.0 a B	0.8 ± 0.0 a A	1.2 ± 0.1 ab C
M Cl			0.9 ± 0.1 a A	0.9 ± 0.1 b A	0.9 ± 0.0 a A	1.1 ± 0.0 a B	0.9 ± 0.0 a A	1.2 ± 0.0 ab B
M Es Cl			0.9 ± 0.1 a BC	1.0 ± 0.0 bc C	0.9 ± 0.0 a AB	1.1 ± 0.0 ab D	0.8 ± 0.0 a A	1.3 ± 0.0 b E
H			1.0 ± 0.1 a B	0.6 ± 0.0 a A	1.3 ± 0.0 c C	1.3 ± 0.1 bc C	1.5 ± 0.0 d D	1.1 ± 0.0 a B
H Bt			1.0 ± 0.1 a B	0.6 ± 0.0 a A	1.1 ± 0.0 b BC	1.2 ± 0.0 ab C	1.5 ± 0.0 d D	1.1 ± 0.0 a B
H Cu			1.0 ± 0.1 a A	0.9 ± 0.1 b A	1.1 ± 0.2 b A	1.6 ± 0.2 c B	1.6 ± 0.0 d B	1.1 ± 0.0 a A
H Bt Cu			1.0 ± 0.1 a AB	0.7 ± 0.0 a A	1.2 ± 0.0 bc B	1.2 ± 0.0 ab B	1.6 ± 0.1 d C	1.3 ± 0.2 ab B

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 27a: Average relative microbial group abundances subtracting reference means in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Gram positive, anaerobic bacteria [%]								
F	0.1 ± 0.2 a DE	0.2 ± 0.1 a E	–0.4 ± 0.1 a BC	–0.4 ± 0.2 a BC	–1.1 ± 0.1 a A	–0.8 ± 0.1 a AB	–0.3 ± 0.2 a CD	–0.3 ± 0.1 ab CD
R	0.0 ± 0.1 a A	0.0 ± 0.0 a A	0.0 ± 0.0 b A	0.0 ± 0.0 bc A	0.0 ± 0.1 bc A	0.0 ± 0.1 cd A	0.0 ± 0.1 ab A	0.0 ± 0.0 c A
M			–0.3 ± 0.1 a A	0.3 ± 0.1 c DE	0.1 ± 0.1 c CD	–0.2 ± 0.1 c AB	0.4 ± 0.0 cde E	–0.1 ± 0.0 c BC
M Es			–0.3 ± 0.1 a A	0.2 ± 0.1 c B	–0.3 ± 0.1 bc A	–0.2 ± 0.1 bc A	0.2 ± 0.1 bc B	–0.2 ± 0.0 bc A
M Cl			–0.3 ± 0.1 a B	0.0 ± 0.1 bc D	–0.1 ± 0.0 bc CD	–0.6 ± 0.1 b A	0.3 ± 0.0 bcd E	–0.2 ± 0.1 bc BC
M Es Cl			–0.3 ± 0.1 a AB	0.0 ± 0.1 bc C	–0.1 ± 0.0 bc BC	–0.4 ± 0.0 b A	0.4 ± 0.2 cde D	–0.2 ± 0.1 bc BC
H			0.2 ± 0.1 b B	0.0 ± 0.1 bc B	0.1 ± 0.1 c B	0.3 ± 0.1 e BC	0.6 ± 0.1 de C	–0.5 ± 0.1 a A
H Bt			0.2 ± 0.1 b B	0.1 ± 0.1 bc B	0.1 ± 0.1 c B	0.1 ± 0.1 de B	0.6 ± 0.0 de C	–0.4 ± 0.0 a A
H Cu			0.2 ± 0.1 b A	0.0 ± 0.2 bc A	–0.6 ± 0.7 b A	0.1 ± 0.1 de A	0.7 ± 0.1 e A	–0.3 ± 0.0 ab A
H Bt Cu			0.2 ± 0.1 b B	–0.2 ± 0.1 ab A	0.1 ± 0.1 c B	0.1 ± 0.1 de B	0.6 ± 0.0 e C	–0.4 ± 0.1 a A
Gram positive, aerobic bacteria [%]								
F	0.0 ± 0.0 a BC	0.0 ± 0.1 a BC	0.0 ± 0.7 a BC	–1.9 ± 0.3 a A	–0.9 ± 0.3 abc AB	–2.0 ± 0.4 a A	–1.4 ± 0.3 a A	0.5 ± 0.4 a C
R	0.0 ± 0.7 a A	0.0 ± 0.6 a A	0.0 ± 0.2 a A	0.0 ± 0.3 a A	0.0 ± 0.2 cd A	0.0 ± 0.1 de A	0.0 ± 0.5 a A	0.0 ± 0.5 a A
M			1.2 ± 1.0 a A	0.2 ± 1.0 a A	0.7 ± 0.1 d A	–0.4 ± 0.4 cd A	0.1 ± 0.4 a A	0.0 ± 0.3 a A
M Es			1.2 ± 1.0 a A	–0.3 ± 0.4 a A	–0.4 ± 0.4 bc A	–0.7 ± 0.2 bcd A	–0.4 ± 0.7 a A	0.1 ± 0.2 a A
M Cl			1.2 ± 1.0 a C	–0.6 ± 0.3 a AB	–0.1 ± 0.4 cd BC	–1.9 ± 0.3 ab A	–0.3 ± 0.3 a ABC	–0.3 ± 0.1 a BC
M Es Cl			1.2 ± 1.0 a A	–0.1 ± 0.3 a A	0.3 ± 0.5 d A	–0.9 ± 0.3 abcd A	–0.3 ± 0.4 a A	0.2 ± 0.2 a A
H			–1.7 ± 0.3 a A	0.3 ± 0.3 a BC	–0.2 ± 0.2 cd B	0.9 ± 0.0 e C	0.0 ± 0.4 a BC	0.9 ± 0.0 a C
H Bt			–1.7 ± 0.3 a A	–0.3 ± 0.8 a A	–1.3 ± 0.6 ab A	–0.6 ± 0.5 c A	–0.7 ± 0.4 a A	–0.1 ± 0.5 a A
H Cu			–1.7 ± 0.3 a A	–0.4 ± 0.9 a A	–1.0 ± 0.3 abc A	–0.8 ± 0.6 bcd A	0.2 ± 0.2 a A	0.4 ± 0.3 a A
H Bt Cu			–1.7 ± 0.3 a A	–0.8 ± 0.25 a A	–1.6 ± 0.3 a A	–1.3 ± 0.5 abcd A	–0.4 ± 0.3 a A	–0.4 ± 0.4 a A
Gram negative bacteria [%]								
F	23.9 ± 1.1 a A	24.3 ± 1.4 a A	1.4 ± 0.7 a A	0.9 ± 0.8 a A	1.1 ± 1.0 a A	0.7 ± 1.4 a A	0.2 ± 1.5 a A	0.4 ± 1.1 a A
R	0.0 ± 0.5 a A	24.4 ± 0.8 a A	0.0 ± 0.8 a A	0.0 ± 0.5 a A	0.0 ± 0.6 a A	0.0 ± 0.4 a A	0.0 ± 1.0 a A	0.0 ± 0.7 a A
M			0.1 ± 0.7 a A	–1.1 ± 1.2 a A	–1.4 ± 0.3 a A	–0.7 ± 0.7 a A	–1.8 ± 0.6 a A	–0.3 ± 0.6 a A
M Es			0.1 ± 0.7 a A	–0.3 ± 0.9 a A	0.2 ± 0.9 a A	0.0 ± 0.5 a A	0.0 ± 1.2 a A	0.2 ± 0.5 a A
M Cl			0.1 ± 0.7 a A	–0.3 ± 0.5 a A	0.0 ± 0.6 a A	0.4 ± 0.8 a A	–0.2 ± 0.6 a A	0.6 ± 0.1 a A
M Es Cl			0.1 ± 0.7 a A	–1.2 ± 0.2 a A	–0.6 ± 0.5 a A	–0.1 ± 0.4 a A	0.1 ± 0.7 a A	0.1 ± 0.5 a A
H			1.0 ± 0.3 a C	–1.3 ± 0.4 a AB	–1.8 ± 0.3 a AB	–2.6 ± 0.8 a A	–1.3 ± 0.8 a AB	–0.2 ± 0.6 a BC
H Bt			1.0 ± 0.3 a A	–0.9 ± 0.9 a A	0.5 ± 1.0 a A	–1.0 ± 1.1 a A	–0.2 ± 0.4 a A	0.7 ± 0.3 a A
H Cu			1.0 ± 0.3 a C	–1.1 ± 0.9 a AB	–0.2 ± 0.3 a ABC	–1.3 ± 0.6 a AB	–1.7 ± 0.3 a A	0.2 ± 0.3 a BC
H Bt Cu			1.0 ± 0.3 a B	–0.7 ± 0.6 a A	0.0 ± 0.5 a AB	–1.0 ± 0.6 a A	–1.2 ± 0.3 a A	1.1 ± 0.3 a B

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

Table 27b: Average relative microbial group abundances subtracting reference means in soils of all treatments during the experiment with standard errors.

Groups	2 weeks	1 week	day of	2 days	1 week	2 weeks	4 weeks	9 weeks
Treatments	before pesticide	application	application	after pesticide application				
Fungi [%]								
F	–0.8 ± 0.3 a AB	–0.1 ± 0.4 a AB	–1.5 ± 0.5 a A	1.0 ± 0.4 a B	0.5 ± 0.5 a B	1.1 ± 0.9 bc B	1.2 ± 1.2 a B	–0.4 ± 0.1 a B
R	0.0 ± 0.4 a A	0.0 ± 0.3 a A	0.0 ± 0.3 bc A	0.0 ± 0.0 a A	0.0 ± 0.2 a A	0.0 ± 0.5 ab A	0.0 ± 0.2 a A	0.0 ± 0.4 a A
M			–0.5 ± 0.7 ab A	0.1 ± 0.8 a A	0.1 ± 0.3 a A	1.1 ± 0.2 bc A	0.9 ± 0.2 a A	0.3 ± 0.1 a A
M Es			–0.5 ± 0.7 ab A	0.1 ± 0.5 a A	0.9 ± 0.7 a A	1.2 ± 0.4 bc A	1.3 ± 0.3 a A	0.3 ± 0.3 a A
M Cl			–0.5 ± 0.7 ab A	0.9 ± 0.1 a B	0.6 ± 0.2 a B	3.1 ± 0.4 d C	0.9 ± 0.3 a B	0.5 ± 0.3 a AB
M Es Cl			–0.5 ± 0.7 ab A	0.6 ± 0.1 a ABC	0.6 ± 0.1 a ABC	1.7 ± 0.3 cd C	1.0 ± 0.4 a BC	0.3 ± 0.4 a AB
H			1.3 ± 0.2 c A	0.7 ± 0.5 a A	0.5 ± 0.8 a A	–0.3 ± 0.5 a A	0.2 ± 0.2 a A	–0.4 ± 0.3 a A
H Bt			1.3 ± 0.2 c A	0.9 ± 0.6 a A	0.4 ± 0.5 a A	1.2 ± 0.4 bc A	0.2 ± 0.1 a A	0.2 ± 0.5 a A
H Cu			1.3 ± 0.2 c C	0.5 ± 0.3 a AB	0.9 ± 0.3 a BC	1.0 ± 0.3 abc BC	–0.2 ± 0.2 a A	–0.2 ± 0.2 a A
H Bt Cu			1.3 ± 0.2 c C	1.1 ± 0.3 a BC	1.1 ± 0.3 a C	1.8 ± 0.4 cd C	0.1 ± 0.3 a AB	0.0 ± 0.4 a A
Actinomycetes [%]								
F	0.1 ± 0.3 a D	0.0 ± 0.2 a CD	–0.6 ± 0.0 a AB	–0.4 ± 0.0 a BC	–0.9 ± 0.1 a A	–0.6 ± 0.1 a AB	–0.1 ± 0.0 a CD	–0.3 ± 0.1 bc BCD
R	0.0 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.2 a A	0.0 ± 0.1 a A	0.0 ± 0.2 c A	0.0 ± 0.1 bcd A	0.0 ± 0.3 a A	0.0 ± 0.1 cd A
M			–0.2 ± 0.1 a A	0.0 ± 0.1 a AB	0.0 ± 0.0 c A	–0.1 ± 0.1 bcd A	0.4 ± 0.2 a C	0.4 ± 0.1 ef BC
M Es			–0.2 ± 0.1 a A	0.2 ± 0.3 a A	–0.2 ± 0.2 bc A	0.0 ± 0.1 bcd A	0.2 ± 0.3 a A	0.6 ± 0.2 f A
M Cl			–0.2 ± 0.1 a AB	0.1 ± 0.2 a AB	–0.2 ± 0.1 bc AB	–0.3 ± 0.1 ab A	0.2 ± 0.1 a BC	0.6 ± 0.1 f C
M Es Cl			–0.2 ± 0.1 a A	0.0 ± 0.1 a A	0.0 ± 0.1 c A	–0.2 ± 0.1 bc A	0.1 ± 0.1 a A	0.2 ± 0.0 de A
H			0.0 ± 0.1 a BC	–0.2 ± 0.1 a AB	–0.1 ± 0.2 bc ABC	0.5 ± 0.0 e D	0.1 ± 0.0 a CD	–0.4 ± 0.1 ab A
H Bt			0.0 ± 0.1 a B	–0.3 ± 0.2 a AB	–0.5 ± 0.1 ab A	0.1 ± 0.2 cd B	0.2 ± 0.1 a B	–0.6 ± 0.2 ab A
H Cu			0.0 ± 0.1 a AB	–0.2 ± 0.1 a A	–0.4 ± 0.3 bc A	0.0 ± 0.1 bcd AB	0.4 ± 0.1 a B	–0.4 ± 0.1 ab A
H Bt Cu			0.0 ± 0.1 a CD	–0.3 ± 0.1 a BC	–0.4 ± 0.1 abc AB	0.2 ± 0.1 de D	0.2 ± 0.2 a D	–0.7 ± 0.1 a A
Protozoa [%]								
F	0.0 ± 0.0 a AB	0.2 ± 0.1 a C	0.1 ± 0.1 a ABC	0.1 ± 0.0 c BC	–0.1 ± 0.0 a A	0.1 ± 0.1 bc BC	0.2 ± 0.0 c C	0.0 ± 0.0 b A
R	0.0 ± 0.0 a A	0.0 ± 0.1 a A	0.0 ± 0.0 a A	0.0 ± 0.0 b A	0.0 ± 0.0 a A	0.0 ± 0.0 ab A	0.0 ± 0.0 b A	0.0 ± 0.1 b A
M			–0.1 ± 0.1 a BC	0.0 ± 0.0 bc D	0.0 ± 0.0 a D	–0.1 ± 0.0 a CD	–0.2 ± 0.0 a AB	–0.2 ± 0.0 ab A
M Es			–0.1 ± 0.1 a BC	0.0 ± 0.0 b C	0.0 ± 0.0 a C	–0.1 ± 0.0 a C	–0.2 ± 0.0 a A	–0.2 ± 0.1 ab AB
M Cl			–0.1 ± 0.1 a BC	0.0 ± 0.1 b C	0.0 ± 0.0 a C	0.0 ± 0.0 a C	–0.2 ± 0.0 a AB	–0.2 ± 0.0 ab A
M Es Cl			–0.1 ± 0.1 a BC	0.0 ± 0.0 bc E	0.0 ± 0.0 a DE	0.0 ± 0.0 ab CD	–0.2 ± 0.0 a A	–0.1 ± 0.0 b AB
H			0.0 ± 0.1 a B	–0.3 ± 0.0 a A	0.4 ± 0.0 c D	0.1 ± 0.1 bc C	0.5 ± 0.0 d D	–0.4 ± 0.0 a A
H Bt			0.0 ± 0.1 a B	–0.3 ± 0.0 a A	0.2 ± 0.0 b C	0.0 ± 0.0 ab B	0.4 ± 0.0 d D	–0.4 ± 0.0 a A
H Cu			0.0 ± 0.1 a AB	0.0 ± 0.1 b AB	0.2 ± 0.2 b BC	0.4 ± 0.2 c C	0.5 ± 0.0 d C	–0.4 ± 0.0 a A
H Bt Cu			0.0 ± 0.1 a A	–0.2 ± 0.0 a A	0.3 ± 0.0 bc BC	0.0 ± 0.0 ab AB	0.6 ± 0.1 d C	–0.2 ± 0.2 ab A

Data followed by various small letters are significantly ($P < 0.05$) different indicating treatment effects (vertical) while data followed by various capital letters are significantly ($P < 0.05$) different indicating temporal effects (horizontal); F: fallow, R: reference, M: mineral fertiliser, Es: endosulfan, Cl: chlorothalonil, H: humus, Bt: Bacillus thuringiensis, Cu: copper oxychloride.

3.2.4.3 Individual microbial taxonomic groups

No significant ($P > 0.05$) differences of absolute PLFA sum contents of the individual taxonomic groups except those of protozoa could be observed both for temporal (Table 24) and treatment effects (Table 25). Consequently, soil microbial community composition as indicated by individual PLFA is also no adequate measure for the differentiation between conventional and organic agriculture. In contrast, Banerjee and Dey (1992) determined a significantly decreasing total number of bacteria, actinomycetes and fungi in rhizosphere microflora of Gangetic alluviums treated with the herbicide basalin and the fungicides dithane and bengard when compared to control soils in a random block design field experiment in West Bengal, India. The authors concluded that different pesticides have different effects on growth and activity of the rhizosphere microflora at various stages of plant growth and thus, they may affect the latter. Contradictory, bacterial and actinomycetes populations in clayey botanical garden soils in Assiut, Egypt, treated with the herbicide brominal and the insecticide selecron were promoted at field application rate but inhibited at fivefold rate (Omar and Abdel-Sater, 2001), while both pesticides significantly decreased the total number of the most determined fungal species. Heavy metal contamination induced a shift of the ratio of fungal to bacterial PLFA in a Calcaric Phaeozem (Kandeler *et al*., 2000) in contrast to the present investigation, in which no effect on this ratio was detectable (data not shown). Kandeler *et al*. (2000) exhibited that heavy metal-resistant fungi can survive in medium polluted soils being supported by the results of Frostegård *et al*. (1996) who found a strong increase of the fungal biomarker PLFA 18:2ω6,9 due to zinc contamination in arable soils in southern Sweden. Nevertheless, in the present investigation protozoa represented by PLFA 20:4ω6 (Cavigelli *et al*., 1995) and three more individual PLFA 14:0, 17:0 and 10Me17:0, which usually indicate all microbial groups and actinomycetes (Zelles, 1999), respectively, exhibited the highest sensitivity against different fertiliser applications. Figure 23 exhibits the PLFA contents subtracted by those of the reference plots in order to eliminate variations caused by climatic factors. Thus, after strong fluctuations at the beginning of the experiment two and four weeks after pesticide application the organic treatments exhibited distinctly higher contents of these PLFA compared to the conventional ones, while at the end of the field experiment the values for the organically managed plots partly decreased dramatically under the level of the conventionally managed ones. Differences caused by the various

pesticide applications were not detectable. Furthermore, plots treated with the Gram positive, aerobic *Bacillus thuringiensis* (Grove *et al*., 2001) did not exhibit higher contents in PLFA a15:0, i15:0 and i16:0 representing Gram positive, aerobic bacteria indicating that it was not possible to quantify the concentration of this biological insecticide in agricultural soils by means of PLFA analysis.

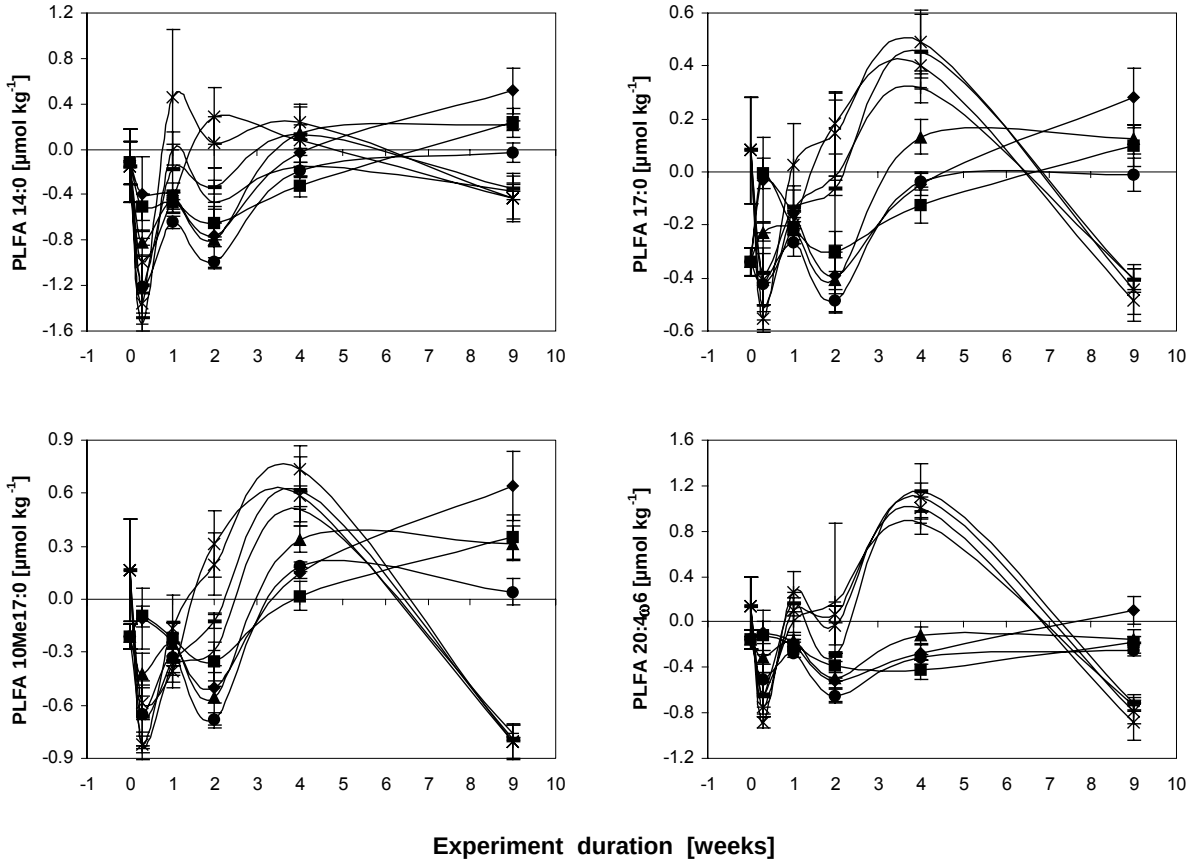

Figure 23: Temporal variation of the individual absolute PLFA contents with standard error bars; ×: only humus; ∗: *Bacillus thuringiensis*; +: copper oxychloride; –: *Bacillus thuringiensis* plus copper oxychloride, ■: only mineral fertiliser, ◆: endosulfan, ▲: chlorothalonil, ●: endosulfan plus chlorothalonil (N = 3); values subtracting those of the reference means.

3.2.4.4 Microbial community composition

Tables 26 and 27 show the relative abundances of the taxonomic microbial groups at the total PLFA content both non-subtracting and subtracting the values of the reference plots, respectively. The Gram positive, anaerobic and the Gram negative bacteria did not exhibit

different trends between the diverse treatments throughout the experiment duration, while the actinomycetes had a significantly ($P < 0.05$) higher relative abundance in conventional treatments than in organic ones at the last sampling day. The latter result was even more obvious when subtracting the relative actinomycete abundance of the reference from those of the diverse treatments at individual sampling dates, especially at the end of the experiment when the values of the organically managed plots tendentially decreased and those of the conventionally managed ones significantly ($P < 0.05$) increased compared to the beginning. Four and nine weeks after pesticide application the relative fungal abundance tended to be higher in conventional treatments in comparison to the organic ones, while the time before no trend was detectable. A clearer development was found in consideration of the Gram positive, anaerobic bacteria, which showed higher relative abundances in organic compared to conventional treatments two and four weeks after the beginning of the experiment, while the values were reversed at the end. The values of the organic treatments mostly decreased and those of the conventional ones increased until the end of the experiment compared to the beginning. The pesticide applications seemed to have no effects on soil microbial community structure, which was in contrast to the results of Smith *et al*. (2000) who determined effects of long-term fungicide applications on microbial properties in tallgrass prairie Udic Argiustolls in northeast Kansas, USA. In that investigation benomyl reduced significantly bacterial biomass and the relative contribution of fungi to total microbial activity. Furthermore, Kelly *et al*. (2003) reported that soils with higher levels of heavy metal contamination showed decreases in indicator PLFA for fungi, Gram positive bacteria and actinomycetes indicating a change in population structure of the soil microbial community resulting from heavy metal contamination. Fraterrigo *et al*. (2006) found that microbial communities in formerly farmed southern Appalachian (USA) forest soils had a higher relative abundance of markers for Gram negative bacteria and a lower abundance of markers for fungi when compared to previously logged and reference stands. Nevertheless, regarding the individual microbial groups again the PLFA 20:4ω6 representing protozoa (Cavigelli *et al*., 1995), the PLFA 10Me17:0 representing actinomycetes as well as the unspecific PLFA 14:0 and 17:0 (Zelles, 1999) exhibited most distinct trends in connection with the fertiliser applications (Figure 24). Equally to the absolute contents, the relative abundances of these PLFA subtracted by those of the reference plots exhibited distinctly higher values in organic than in conventional

treatments two and four weeks after field experiment beginning, while those of the latter treatments reduced drastically at the end. Again differences caused by the various pesticide applications were not detectable.

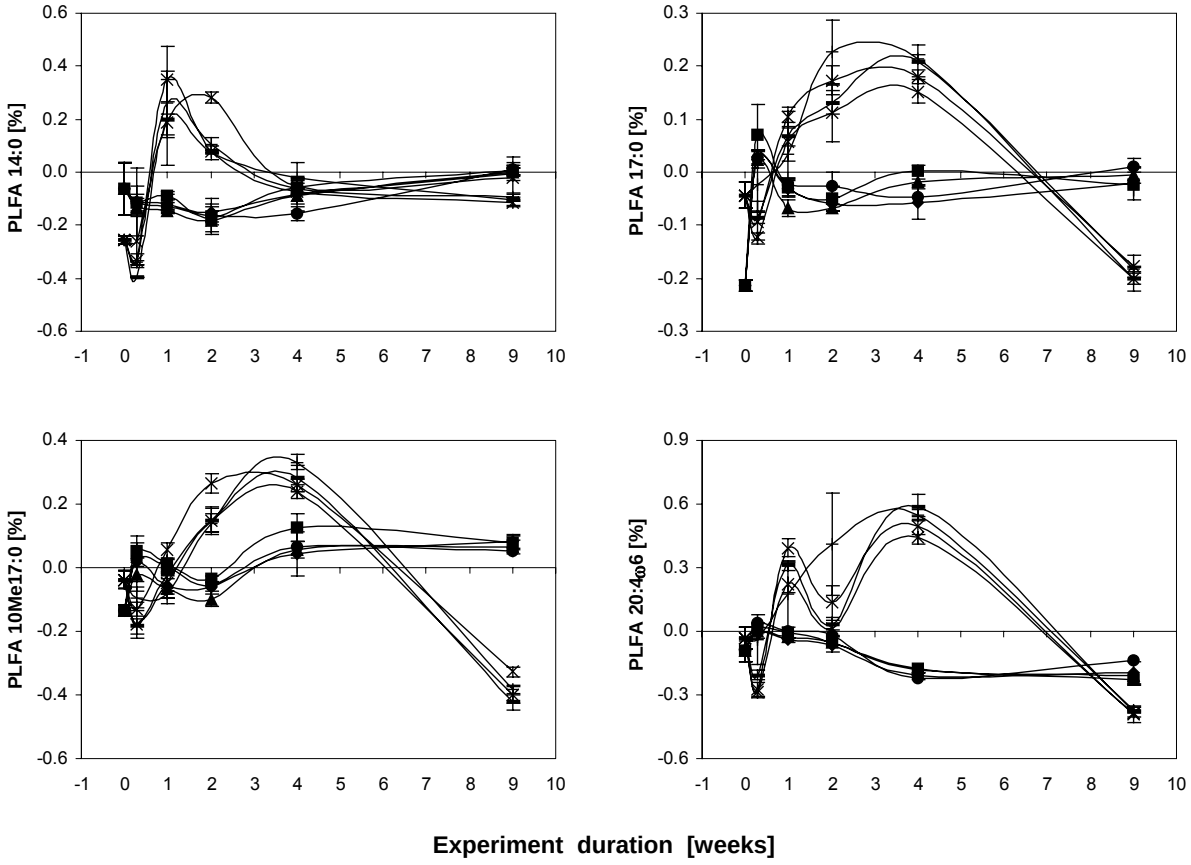

Figure 24: Temporal variation of the individual relative PLFA abundances with standard error bars; ×: only humus; ∗: *Bacillus thuringiensis*; +: copper oxychloride; –: *Bacillus thuringiensis* plus copper oxychloride, ■: only mineral fertiliser, ◆: endosulfan, ▲: chlorothalonil, ●: endosulfan plus chlorothalonil (N = 3); values subtracting those of the reference means.

3.2.4.5 Discriminant analysis

Although it was not possible to attribute the principal components to specific microbial taxonomic groups it was reasonable to compute the discriminant analysis with the factor scores obtained by the principal component analysis in order to investigate whether it is possible to distinguish between soils of different management systems. This was done in the style of the discriminant analysis of the soil microbial community functional parameters (see 3.2.3.5, page 106), in which it was feasible to differentiate between conventional and organic

treatments as well as fallow and reference plots within the field experiment. The present results derived from individual microbial groups and microbial community composition that pesticides caused no effects on PLFA pattern could be explained by a low bioavailability of pesticides in soils with high organic carbon and high clay contents (Ahtiainen *et al*., 2003), which may also be the case for the Mollisol under study. Hence, the data of the plots treated with pesticides were omitted in this analysis at first. Soils of the same treatments such as fallow, reference, conventionally and organically fertilised plots at the same sampling day were expected to be allocated in the respective discriminant group. 94.7 – 100.0 % of the total variance of all group centroids were described by the first (54.5 – 94.6 %) and the second (5.2 – 40.2 %) canonical discriminant functions (CDF) and canonical correlation coefficients close to 1 (CDF 1: 0.964 – 0.993 and CDF 2: 0.852 – 0.964) indicated strong correlations between discriminant scores and groups. Very low Wilks' Lambda values close to 0 (CDF 1: 0.001 – 0.005 and CDF 2: 0.041 – 0.240) exhibited that the group means were different, which was confirmed by a high significance greater than 87.5 %. The third canonical discriminant functions, which explained 0.0 – 5.3 % of the total variance were inconsiderable. In Figure 25 the first two resulting canonical discriminant functions for all sampling days are plotted and it was obvious that a differentiation among the treatments of different fertilisation and the degree of land use was predominantly possible. A distinct differentiation from the other treatments was observable for the fallow plots and those of the organically managed ones. Congruent to the trend of the pH value (Figure 16, page 78) at the first four sampling days a difference between reference plots and those of the conventional management was not detectable, while at the end of the experiment also these diverse treatments were clearly differentiable. Consequently, in comparison to the investigation of the functional parameters it could be concluded that the soil microbial community structural parameters were less sensitive against soil alteration than soil microbial community functional parameters since with the aid of the latter ones it was already possible to distinguish between the various treatments at the day of pesticide application. The same kind of discriminant analysis was also applied to the data of the conventionally and organically managed plots whereas it was investigated whether the plots only treated with mineral fertiliser as well as those plus endosulfan, plus chlorothalonil or plus both pesticides and the plots only treated with humus as well as those plus *Bacillus thuringiensis*, plus copper oxychloride or plus both

pesticides, respectively, are differentiable. The analyses exhibited that it was neither possible to differentiate between soils treated and non-treated with pesticides nor among soils treated with one or two applications (data not shown). Consequently, differences in soil microbial community structure were only induced by various fertiliser additions, which confirmed the same results of the investigation of the soil microbial community function.

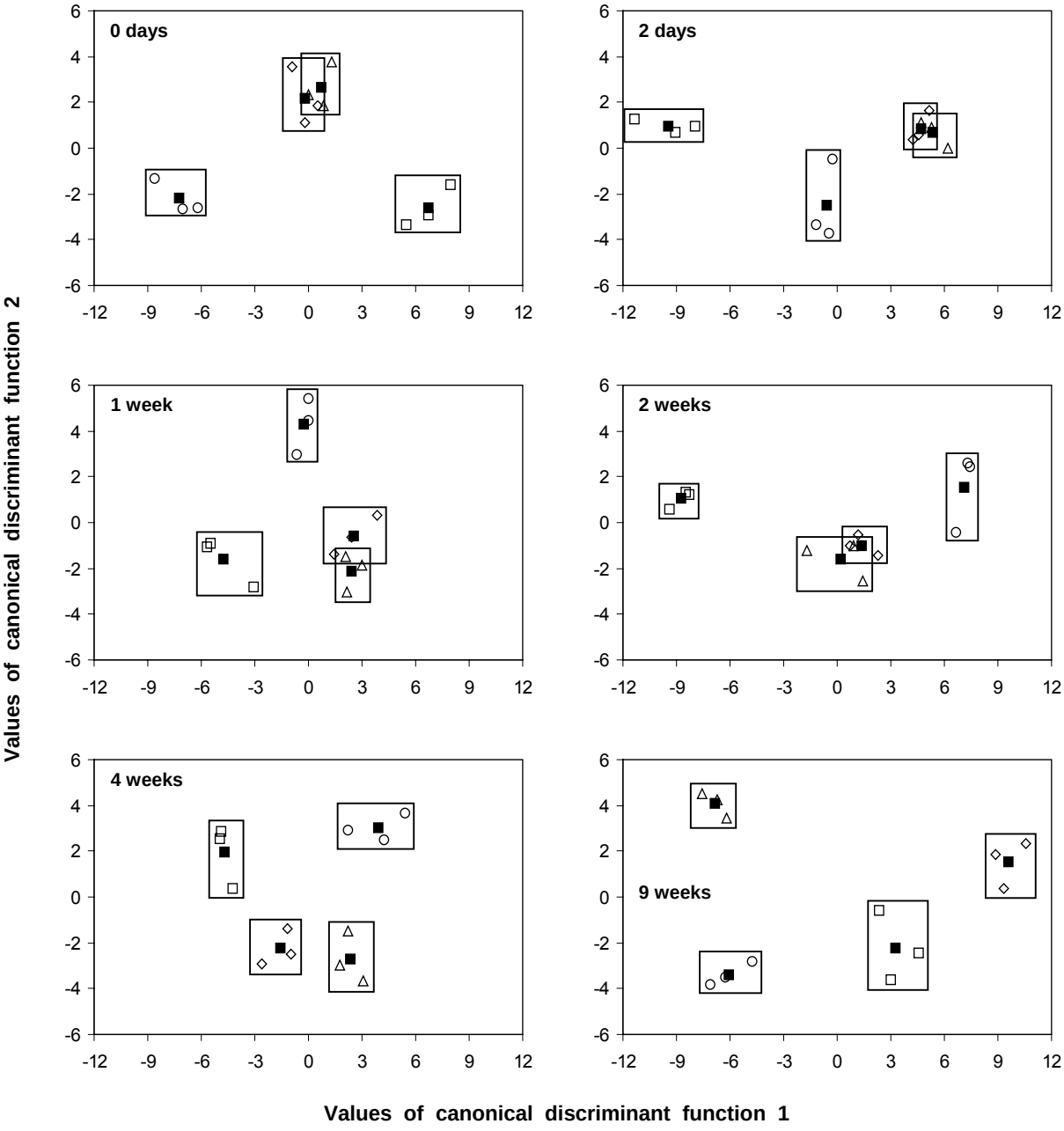

Figure 25: Chronological sequence of the values of the canonical discriminant functions 1 and 2 at all sampling dates after pesticide application; □: fallow, ◇: reference field, △: conventional field, ○: organic field, ■: group centroids.

3.2.5 Linking soil microbial community structure to function

Since the microbial community structure is not sufficient as an indicator for soil quality (Schloter *et al*., 2003b) absolute PLFA contents of the microbial taxonomic groups influencing ecosystem processes were correlated with the microbial community functional parameters. Table 28 exhibits correlation coefficients and significance levels between the structural and functional parameters. Equal to the total PLFA content – the measure of viable microbial biomass – all microbial group contents correlated highly significantly ($P < 0.05$) with the arylsulfatase activity and the soil microbial biomass content, which was computed from the substrate-induced respiration. This result emphasised the significance of the two parameters being in the first principal component of the principal component analysis of the functional parameters and representing the microbial capacity. By contrast, with the exception of the protozoa all microbial taxonomic groups mostly exhibited highly significant ($P < 0.05$) negative correlations with the metabolic quotient indicating microbial growth without stress. The metabolic quotient reflects the physiological status of the soil microbial community and may be used as a stress indicator for ecosystems (Jones *et al*., 2001). According to this, the lower the value of this quotient is the higher is the microbial biomass and the lower is the basal respiration. Consequently, the microbes did not correlate with the substrate-induced respiration because the easily degradable carbon sources offered during this analysis were used for production of biomass and less of energy. Contradictory to the study of the land use systems, in which the protozoa were the microbial group providing the lowest ecological significance, in the field experiment, this microbial group exhibited the most mainly highly significant ($P < 0.05$) correlations with the functional parameters and thus, it was the most sensitive group against soil alteration. This result was confirmed by the absolute contents and relative abundances of protozoa (Figures 23 and 24, page 127 and 129, respectively) showing an obvious differentiation between organically and conventionally managed plots in comparison to the abundances of the remaining groups. However, when compared to the results of the land use systems this situation seemed to be reversible because after some time when all microbial groups adapted to soil alteration the protozoa apparently loose ecological significance. The Gram positive bacteria including actinomycetes and the Gram negative bacteria exhibited fewer correlations when compared to the previous study indicating that these groups were inhibited by soil alteration and had to cope with the new soil conditions at

first. However, fungi, which are considered more resistant to stress and soil alteration (Bååth and Anderson, 2003; Kandeler *et al.*, 2000) maintained their ecological significance despite new habitat conditions and exhibited even more correlations to functional parameters as in actual agricultural land use system soils. Beneath changed soil physico-chemical properties particularly caused by tillage and fertiliser application but hardly because of pesticide treatments recently arisen ecological niches were not occupied by specific microbial groups, yet. According to this and the principal component analysis, in which a classification into microbial taxonomic groups was impossible, the most correlations were found with the same functional parameters since no specification into various ecological tasks took place. Hence, the correlation analysis of the field experiment samples reflected soil microbial conditions immediately after soil alteration, while that of the investigation of the different land use systems described the microbial status of soils being in ecological equilibrium.

Table 28: Correlation coefficients between PLFA contents of the microbial taxonomic groups and soil microbial functional parameters (N = 177).

Soil microbial functional parameters	Gram +, anaerobic b.	Actino-mycetes	Protozoa	Gram - bacteria	Gram +, aerobic b.	Fungi
Acid phosphatase	0.250 **	0.192 *	0.383 **	0.081	0.051	0.192 **
Arylsulfatase	0.367 **	0.290 **	0.325 **	0.276 **	0.246 **	0.231 **
Cellulase	-0.080	-0.094	0.023	0.021	-0.068	0.023
Dehydrogenase	0.130	0.071	-0.247 **	0.280 **	0.221 **	0.152 *
Urease	-0.035	-0.055	0.100	-0.059	-0.147	-0.075
Basal respiration	0.201 **	0.120	0.297 **	0.247 **	0.110	0.227 **
Substrate-induced respiration	0.132	0.067	-0.137	0.072	0.139	0.138
Microbial biomass	0.514 **	0.444 **	0.247 **	0.452 **	0.340 **	0.511 **
Metabolic quotient	-0.280 **	-0.296 **	0.016	-0.188 *	-0.216 **	-0.265 **
Net nitrogen mineralisation	0.160	0.157	0.014	0.038	0.045	0.188 *
Net nitrification	0.066	0.084	-0.290 **	0.066	0.127	0.118
Potential denitrification	0.009	-0.152 *	0.149 *	0.085	-0.092	0.016

* significant ($P < 0.05$), ** highly significant ($P < 0.01$); b.: bacteria.

4. Conclusions

Soil scientists, agronomists and ecologists are concerned about the effects of continuous intensive soil management practices on the diversity of soil microbial communities and on soil sustainability. On the one hand, in this dissertation the anthropogenic stress resulting from different agricultural land use systems on microbial community structure and function in soils of conventional and organic cultivation on open agricultural fields as well as under greenhouses, pastures and fallows of the inner zone of the green belt around Buenos Aires, Argentina, was characterised. Additionally, soils of an anthropogenically unaffected natural site was used as a reference. On the other hand, a field experiment was designed in order to examine the suitability of microbial parameters to differentiate between recently established agricultural management systems (conventional versus organic) in a land use conversion starting from a fallow with particular respect to pesticide and fertiliser application. Hence, the pesticide and heavy metal contamination situation was examined in topsoils of the investigation area. Moreover, soil microbial community function was determined by enzyme activities (acid phosphatase, arylsulfatase, cellulase, dehydrogenase and urease), basal as well as substrate-induced respiration, microbial biomass, metabolic quotient, net nitrogen mineralisation, net nitrification and potential denitrification, while soil microbial community structure was studied by means of phospholipid fatty acid (PLFA) analysis.

4.1 Land use systems

The pesticides carbofuran, chlorpyrifos, chlorothalonil, dimethoate, endosulfan (α- and β-isomer and the metabolite endosulfan sulfate), permethrin and trifluralin were determined exclusively in soils of conventionally managed fields or in greenhouses and in one fallow soil, which was previously also conventionally managed. Therefore, only on conventional sites chemical pest control was performed and thus, conventionally managed fields could be distinguished from other land use system soils by pesticide analysis. Pesticides below the detection limit were cypermethrin, deltamethrin, malathion and triazofos. Hence, no risk potential was expected from pesticide contamination for environment since the determined pesticide concentrations in soils of the conventionally managed fields were distinctly below the intervention values of the Dutch List. Low pesticide concentrations could be caused by volatilisation, leaching, erosion, mineralisation or fixation to soil particles as bound residues.

The fact that no pesticides were found in the non-conventional sites implies that the risk for human health via soil erosion and / or food contamination can be considered as low. Copper, lead, manganese, nickel, iron and zinc could be quantified in all soil samples, while cadmium could only be detected in one fallow soil. As no significant differences in heavy metal contents in the investigated soils including the reference site could be observed no significant anthropogenic heavy metal sources such as pest control, mineral phosphate fertilisers or sewage sludge could be assumed. Additionally, atmospheric contamination caused by local industry and traffic could be considered as low when compared to reference values (e.g. Dutch List). For this reason, it was not possible to separate land use systems by heavy metal contents. All heavy metal levels were within the background range for uncontaminated soils. Hence, heavy metal contents were not regarded as harmful for the environment. Unfortunately, no investigations of heavy metal and pesticide contamination of agricultural soils in other metropolitan areas with the same socio-economic background as described for Buenos Aires such as São Paulo (Brazil), Bangkok (Thailand) or Kathmandu (Nepal) were found for the purpose of comparison to the present study.

Since the examination of individual soil microbial community functional parameters did not provide unambiguous results these parameters were divided into the following four components via principal component analysis:

- Microbial capacity (microbial biomass carbon, acid phosphatase, arylsulfatase and dehydrogenase activities),
- Mineralisation activity (cellulase activity, substrate-induced respiration, net nitrogen mineralisation and net nitrification),
- Nitrogen transformation potential (urease activity and potential denitrification),
- Metabolic activity (basal respiration and metabolic quotient).

By means of a subsequent discriminant analysis the soils of the reference, pastures as well as greenhouses and organically managed agricultural fields could be separated. Only soils of the conventionally managed agricultural fields and the fallows could not be differentiated indicating insufficient time for conversion of microbial parameters. With the knowledge that all fallow soils previously have been conventionally cultivated the assumption that both land use systems exhibit similar soil microbial function properties was clarified by a hierarchical cluster analysis. Additionally, it was exhibited that not cultivation systems were mainly

responsible for differences in properties of agriculturally managed soils but factors resulting from indoor or outdoor cultivation. Furthermore, the reference and pasture soils were very different to those of the agricultural managements. The reference soils showed much higher values for basal respiration and microbial quotient compared to the other land use systems, while the pasture soils exhibited a higher microbial biomass and increased enzyme activities.

With the aid of relative abundances, individual PLFA could be divided into the following four components representing particular taxonomic groups via principal component analysis and literature corresponding to their highest factor loadings:

- Principal component 1: Gram positive, anaerobic bacteria (PLFA cy17:0, a17:0, i17:0), actinomycetes (PLFA 10Me17:0, 10Me18:0) and protozoa (PLFA 20:4ω6).
- Principal component 2: Gram negative bacteria (PLFA 16:1ω5c, 16:1ω7c, 18:1ω7c).
- Principal component 3: Gram positive, aerobic bacteria (PLFA a15:0, i15:0, i16:0).
- Principal component 4: Fungi (PLFA 18:1ω9c, 18:2ω6,9).

Total PLFA contents revealed that any agricultural land use led to a decrease in microbial biomass in comparison to anthropogenically unaffected soils. The lowest biomass reduction was detected in pasture soils because of organic matter input through excrements of animal stocks. Soils of greenhouse cultivations exhibited tendentially lower contents compared to outdoor cultivations on agricultural fields. Gram negative bacteria and fungi showed higher contents in soils with organic managements than in those with conventional ones. Fallow soils, which mainly had the lowest contents in all soils for every taxonomic group, indicated that soil microorganisms need a long time to recover from agricultural land use regarding that these soils have not been used at least for two years. Most of the microbial taxonomic groups relatively decreased compared to the reference soil – in particular the Gram positive, aerobic bacteria. Fungi and Gram negative bacteria relatively increased although they decreased in absolute terms. Therefore, a change of soil microbial community composition was obvious because of any agricultural land use. In soils of conventionally and organically managed agricultural fields and fallow sites, Gram negative bacteria exhibited significantly greater contents than soils of the remaining land use systems. This might be caused by better aeration due to higher tillage activity and resulting in lower soil density on the one hand or due to other climatic conditions or irrigation practice on the other hand in indoor compared to outdoor cultivations.

It can be concluded that the current organically and conventionally agricultural practice of Argentinean farmers concerning plant protection and fertilisation in the inner zone of the green belt around Buenos Aires is not considered as endangering or alarming for human health, soil and water quality, plant growth and wildlife. Nevertheless, the development of agriculture in this area has to be observed further on in case the situation comes to a head because the pressure on agriculture will increase due to continuing population growth, city expansion and soil sealing. This investigation clearly showed that no significant differences in microbial parameters were detected neither between soils of conventional and organic management systems nor between cultivations on agricultural fields and greenhouses using individual microbial parameters. Instead, it was clearly demonstrated that it was possible to distinguish between various agricultural medium- to long-term (> 2 years) land use systems using a set of microbial functional parameters followed by multivariate statistical analysis. However, this investigation revealed that the selected soil microbial functional parameters were not sensitive enough for short-term (< 2 years) land use changes. Furthermore, it can be stated that PLFA analysis is a valuable tool for evaluating soil microbial diversity. However, this diversity and the microbial composition of the soils under study were not significantly influenced by the different agricultural land use systems. Therefore, further investigations should be performed in order to find other parameters, which are regarded as spontaneous indicators of variations in soil physical, chemical or biological properties for the detection of land use changes on the short term and for the examination how sensitive the presented procedures are.

4.2 Field experiment

Resembling the land use systems the examination of individual soil microbial community function parameters of the field experiment did not provide unambiguous results. Therefore, these parameters were also divided into four components via principal component analysis:

- Microbial capacity (microbial biomass, acid phosphatase and arylsulfatase activity),
- Mineralisation activity (substrate-induced respiration, dehydrogenase and urease activity, net nitrogen mineralisation and net nitrification),
- Metabolic activity (basal respiration and metabolic quotient),
- Nitrogen transformation potential (potential denitrification).

The cellulase activity could not be taken into account because of too low correlation coefficients to the principal components indicating a too low contribution at the whole soil microbial functionality in this field experiment. With the aid of a subsequent discriminant analysis the plots of the fallow, reference and conventional as well as organic treatments could be separated at any sampling date throughout the experiment duration. By contrast, it was not possible to differentiate between soils treated with pesticides and only fertilised ones both in conventionally and organically managed treatments. Equally no differences were found in soils treated with one or two pesticides. Consequently, differences in the activity of the soil microbial functions were only induced by various fertiliser applications.

Phospholipid fatty acid (PLFA) profiles actually provide a robust measure, which can be used to fingerprint the structure of soil microbial communities and to calculate their biomass. On the one hand, probably due to a still too homogeneous data set (same soil and recent changes in management practice) a differentiation of soil microbial community structure by means of a principal component analysis was not possible. On the other hand, with the aid of a subsequent discriminant analysis it was possible to distinguish between soils of non-fertilised agricultural field, fallow as well as conventionally and organically managed plots, indicating small differences among individual microbial groups but synergistic effects of soil microbial community structure. However, a differentiation between the non-fertilised and conventional treatments was only feasible for six weeks after fertiliser addition to the soil. In addition, a discrimination among soils of the same management system being treated with various pesticides was not feasible. Furthermore, a quantification of the amount of applied Gram positive *Bacillus thuringiensis* in organic cultivation by means of PLFA analysis was not possible as opposed to the other pesticides, which could be determined by residue analysis. Four PLFA (14:0, 17:0, 10Me17:0 and 20:4ω6) were identified being very sensitive to humus or mineral fertiliser application regarding both absolute and relative abundance.

In literature there are several studies showing strong effects of pesticides on soil microbes by application of two to ten times higher pesticide concentrations as commercially used. The present investigation was consciously conducted using actual pesticide amounts in order to determine the current impact of Argentinean agriculture on the structure and function of the soil microbial community during a single vegetation period. According to the present results mentioned above, the actual agricultural practice does not drastically influence the activity of

soil microorganisms and thus soil sustainability. These results indicate that the clayey and silty as well as fertile Mollisols of the investigation area constitute very stable soil ecosystems potentially exhibiting a low bioavailability of pesticides. On this account, it was not possible to state a quality index for these soils. Additionally, many correlations between soil microbial community structure and function parameters were found in both parts of this dissertation indicating close connections between soil microbial community structure and function. Therefore, microbial community composition alone was of no indicator value for soil quality and had to be combined with functional properties.

To draw a conclusion, a gentle utilisation of the valuable resource soil is crucial for sustainable agriculture in mega-cities such as Buenos Aires. However, since soil microbial community structure and function provide details about the integrated state of soil quality and disturbance regimes the evaluation of sustainability of intensely agriculturally used soils is not only of interest in mega-cities. The results of this investigation can be used for giving advices for sustainable agriculture in the periurban area of Buenos Aires and can provide basic knowledge about other conurbations in the world. With special regard to the situation in the surroundings of Buenos Aires, according to the results of the present dissertation, under current circumstances soil degradation and contamination as well as disturbance of soil community structure and function are not expected. Admittedly, the situation in several years of continuing land use intensification is arguable why long-term observations and investigations on more severe soil conditions are required and suggested for the future.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet habe.

Ferner erkläre ich, dass ich weder an der Universität Bayreuth noch anderweitig versucht habe, mit oder ohne Erfolg eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

Bayreuth, 17. Juli 2007

(T. Ullrich)