Interactions of organic amendments, pea (*Pisum sativum* L.) growth, arbuscular mycorrhizal fungi and other soil microorganisms

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Dissertation

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by

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Witzenhausen Juni. 2016

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Ramia Jannoura

Dedication

To *my parents* for their love and encouragment

To *my Husband* for his patience and sacrifice and love

To my two beautiful *Daughters* “Grace” and “Naya” who brighten my life.

Preface

The thesis is submitted to the Faculty of Organic Agricultural Sciences -Department of Soil Biology and Plant Nutrition to fulfil the requirements for the degree "Doktor der Agrarwissenschaften" (Dr. agr.). The cumulative dissertation is divited into five parts. The manuskripts are included in chapters 4, 5, 6 and 7 are based on four papers as first author, which are published in international refereed journals. In the chapter 3 is a greenhouse experiment, which was listed as a preliminary experiment in this work, this experiment was repeated again (in chapter 4) for scientific reasons mentioned later. Chapter 1 is the general introduction to the thema, while the chapter 2 describs the objectives of this work. Chapter 8 summarizes the results of all the chapters together, and is listed in deutsch in chapter 9. Chapter 10 contains the conclusion and outlook. Supplymantary materials are found in chapter 11.

The following papers are included in this thesis:

Chapter 4

Jannoura, R., Kleikamp, B., Dyckmans, J., Joergensen, R.G., 2012. Impact of pea growth and of arbuscular mycorrhizal fungi on the decomposition of ^{15}N- labeled maize residues. Biology and Fertility of Soils 48, 547–560

Chapter 5

Jannoura, R., Bruns, C., Joergensen, R.G., 2013. Organic fertilizer effects on pea yield, nutrient uptake, microbial root colonization, and soil microbial biomass indices in organic farming systems. European Journal of Agronomy 49, 32-41.

Chapter 6

Jannoura, R., Joergensen, R.G., Bruns, C., 2014. Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. European Journal of Agronomy 52, 259-270

Chapter 7

Jannoura, R., Brinkmann, k., Uteau, D., Bruns, C., Joergensen, R.G., 2015. Monitoring of crop biomass using true color aerial photographs taken from a remote controlled hexacopter. Biosystems Engineering 129, 341-351.

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List of abbreviations

%Ndfa	Plant nitrogen derived from N_2 fixation
‰	Promille
Al	Aluminium
AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
ANOVA	analysis of variance
BBCH	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie
C	Carbon
C3-plant	Plant with C3 pathway for carbon fixation in photosynthesis
C4-plant	Plant with C4 pathway for carbon fixation in photosynthesis
Ca	Calcium
$CaCl_2$	Calcium chloride
CFA	continuous flow analyser
CFE	Chloroform fumigation extraction
$CHCl_3$	Chloroform
CIRAS	Combined infrared gas analysis system
cm	Centimeter
C_{mic}	Microbial biomass carbon
CO_2	Carbon dioxide
CV	Coefficient of variation
cv.	Cultivar
DAS	Days after sowing
DW	Dry weight
Eq.	Equation
FAO	The Food and Agriculture Organization of the United Nations
Fe	Iron
Fri	FRISSON
GCP_S	Ground control points
GRVI	Green-red ratio vegetation index
h	Hour
H^+	Hydrogen ion
H_2O	Water

H_2O_2	Hydrogen peroxide
HCl	Hydrochoric acid
HCO_3^-	Hydrogencarbonate
HI	Harvest index
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
K_2SO_4	Potassium sulphate
k_{EC} , k_{EN} and k_{EP}	Extractable part of the total amount of carbon (k_{EC}), nitrogen (k_{EN}) and phosphorus(k_{EP}) bound in the microbial biomass
KH_2PO_4	potassium dihydrogen phosphate
KOH	Potassium hydroxide
KWS	Klein Wanzlebener Saatzucht (seed breeding from Klein Wanzlebener)
LAI	Leaf area index
LER	Land equivalent ratio
LSD	Least significant difference
m	Meter
M	Molarity
mm	Millimeters
Mg	Magnesium
Mn	Manganese
myc$^-$	The non-mycorrhizal isoline/ mutant P2
myc$^+$	The symbiotic isoline Frisson
min	Minute
N	Nitrogen
n.d.	Not determined
N_2	Gaseous nitrogen
Na	Sodium
NaCl	Sodium chloride
$NaHCO_3$	sodium hydrogen carbonate
ND	Digital Nummbers
NDVI	Normalised Difference Vegetation Index
NGRDI	Normalised Green–Red Difference Index
NH_4^+	Ammonium
NH_4NO_3	Ammonium nitrate
NIR	Near-infrared

NO_3	Nitrate
N_{mic}	Microbial biomass nitrogen
no.	Numbers
nod^-	non-nodulating isoline
nod^+	Nodulating isoline
NS	Not significant
P	Phosphorus
P2	Isogenetic mutant P2 of variety FRISSON
ppm	Parts per million
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PH	Potential Hydrogen
P_{mic}	Microbial biomass phosphor
POM	Particulate organic matter
Rev	Revolutions
r	Spearman correlation coefficient
RGB	Red green and blue colour
S	Sulfur
sec	second
t	Tonne
ICP	Inductively Coupled Plasma
UAV	Unmanned aerial vehicle
VI	Visible vegetation index
Vol.	Volume
yr	year
w/w	Weight ratio
WHC	Water holding capacity
WRB	World Reference Base for Soil Resources
$\delta^{13}C$	$^{13}C/^{12}C$ ratio expressed relative to the PDB standard
$\delta^{15}N$	Stable nitrogen isotope signature

1. General introduction

The world population will grow from the present 7 billion people to 8 billion by 2025 and to almost 9 billion by the year 2043 and most of this growth will occur in developing countries (United Nations, 2011). With the limitation on arable land area, the productivity per unit of land has to be increased to meet the requirements of the growing population for food, fodder and fiber. Many intensive farming practices, including continuous cropping and heavy applications of fertilizers and pesticides, have been attempted with the aim of achieving high crop yields. However, they often contribute to severe environmental deterioration, degradation of soil quality, and loss of biological diversity of the soil (Mäder et al., 2002). The major challenge is how to increase crop productivity while maintaining or even enhancing the levels of soil organic matter and soil fertility.

Soil fertility, the ability of a soil to supply nutrients to growing plants, depends on physical, chemical, and biological soil attributes and plays a crucial role in determining the productivity of all agricultural systems (Jeffries et al., 2003). The maintenance of soil fertility depends on the size and the activity of the soil microbial biomass (Jeffries et al., 2003), which has been used as an index of soil fertility (Singh et al., 2007). Soil microorganisms play a major role in regulating soil biological processes such as decomposition of organic matter and nutrient cycling (Böhme et al., 2005). Today, particular interest is focused on the importance of the soil microbial biomass in relation to soil fertility and crop nutrition (Goyal et al., 1992; Saini et al., 2004).

Microbial decomposition of organic substances in soil such as plant residues or organic fertilizer is a central process for maintaining nutrient cycling, and it can be affected by the presence of plants, especially by mycorrhizal plants, in different ways (Pare et al., 2000; Hodge et al., 2001). In agricultural ecosystems, a specific feature of most crop and grassland species, especially of legumes, is the presence of arbuscular mycorrhizal fungi (AMF), which transfer between 5% to 20% of plant assimilates into the soil (Jones et al., 2001; Fitter et al., 2011). AMF play a central role in ecosystem functioning by influencing nutrient fluxes and their interaction with other microorganisms in the rhizosphere (van der Heijden et al., 2008). Recently it has been reported that AMF can both enhance decomposition and increase nitrogen capture from complex organic matter in soil (Hodge et al., 2001).

There is an increasing awareness of environmental damage caused by the use of non-renewable chemical resources in agriculture. Therefore, a huge research effort has been directed towards alternatives such as organic farming, which is rapidly expanding worldwide (Willer and Kilcher, 2011). Several studies have shown that organic management leads to higher soil quality with higher microbiological activity than conventional farming (Marinari et al., 2006). In organic agricultural, where the use of chemically synthesized fertilizers, pesticides, herbicides and fungicides is prohibited, legume crops such as peas (*Pisum sativum* L.) and organic fertilizer application play a particularly important role in maintaining soil fertility. The nutrient value of organic manures can be significant, but varies considerably depending on origin, treatment before application, and the content of straw or other bedding material (Sørensen et al., 1994; Thomsen and Kjellerup, 1997).

Also, an important form of agriculture and very suitable for organic farming is the use of intercropping strategies. Intercropping is widely practiced as a sustainable agriculture system, due to its yield advantage and high utilization efficiency of environmental sources such as light, water, and nutrients (Andersen et al., 2004). In particular, legume-cereal intercropping systems offer a potential method to reduce the dependence on industrial N input by increasing symbiotic N_2 fixation (Hauggaard-Nielsen et al., 2001). However, few studies have focused on the microbial properties in the rhizosphere of intercropping systems, which may account for its yield advantage (Song et al., 2007).

The use of unmanned aerial vehicles has been increasing in precision agriculture recently as an alternative to very costly and not readily available satellites or airborne sensors. The use of small rotary wing UAVs to remotely detect crop and soil properties may become a key factor for farmers in the future, with promising capabilities for remote sensing applications in agriculture.

1.1. Importance of pea (*Pisum sativum* L.)

Pea (*Pisum sativum* L.) is one of the most important food legume crops, based on world production estimates (FAO, 2013). In 2013, globally, green and dry peas were grown on 2.3 and 6.7 million ha, with average productions of 17.4 and 11.4 million tons, respectively. The major producing countries of green pea are China (60% of global production), India (23%) and of dry pea are Canada (35%), China (14%) and the

Russian Federation (12%) (FAO, 2013). Major pea producing countries in the world are listed in Table 1.1.

Pea is a cool season crop grown in many parts of the world for human nutrition and animal feed. Peas are rich sources of proteins (25%), carbohydrates (more than 50%), vitamins, and several mineral elements such as potassium, phosphorus and calcium (de Almeida Costa et al., 2006). In organic farming systems where biological N_2 fixation is the main source of N input, the ability of legumes, such as peas, to fix atmospheric N_2 is of particular importance (Berry et al., 2002). Under field conditions, pea can obtain a large proportion (approximately 59-72%) of its N from biological fixation (Peoples et al., 2009), fixing up to 250 kg N ha^{-1} yr^{-1} (Karpenstein-Machen and Stuelpnagel, 2000). Pea is an important rotation crop in many parts of the world, contributing a substantial amount of nitrogen to the subsequent non-legume crops (Stevenson and van Kessel, 1996). Pea also can be incorporated into the soil as green manure (Biederbeck et al., 1996). A major problem of growing pea as a sole crop is the high degree of yield variability (Jensen, 1996), due to several factors such as drought sensitivity, lodging, and weak competitive ability towards weeds (Jensen, 1996; Mcdonald, 2003; Hauggaard-Nielsen et al., 2008). However, semi-leafless varieties proved to be less susceptible to lodging than conventional leafed varieties, and less sensitive to drought because of their reduced surface leaf area (Baigorri et al., 1999), but their competitiveness against weeds is low (Sauke and Ackermann, 2006).

Table1.1**:** Major pea-producing countries in the world ranked by production, 2013

	Peas, Dry		Peas, Green	
	Country	Production (t)	Country	Production (t)
1	Canada	3960800	China	10606000
2	China	1566000	India	4006200
3	Russian Federation	1350167	United States of America	323004
4	United States of America	708510	France	228987
5	India	600000	Algeria	186348
6	France	498940	Egypt	169122
7	Ethiopia	379813	United Kingdom	152570
8	Ukraine	267240	Peru	130065
9	Australia	239750	Morocco	125482
10	Iran	195000	Pakistan	114033

Source: FAO statistical data; FAOSTAT database, t (tons)

1.2. Mycorrhizal plant and the decomposition process:

Mycorrhizae are symbiotic associations between soil fungi and the roots of most vascular plants (Smith and Read, 2008). There are two main types of mycorrhizal associations; ectomycorrhizae, where fungi proliferate between root cells without entering the interior of the cells, and endomycorrhizae, where fungi penetrate the root cell walls (Bolan, 1991; Smith and Read, 2008). The most widespread mycorrhizal associations belonging to the endomycorrhizal fungi are AMF, which colonize more than 80% of the higher plant species (Smith and Read, 2008).

The initiation of the AM association begins with the germination of spores and hyphal development; subsequently, the hyphae penetrate the roots of host plants and develop within the cortex cells, forming arbuscules and/or vesicles (Strack et al., 2003) (Fig. 1.1 and 1.2). The arbuscules are responsible for nutrient exchange between the host and the symbiont, transporting carbohydrates from the plant to the fungus, and mineral nutrients from the fungus to the plant (Strack et al., 2003). Vesicles are considered to be storage organs, mainly containing lipids (Smith and Read, 2008).

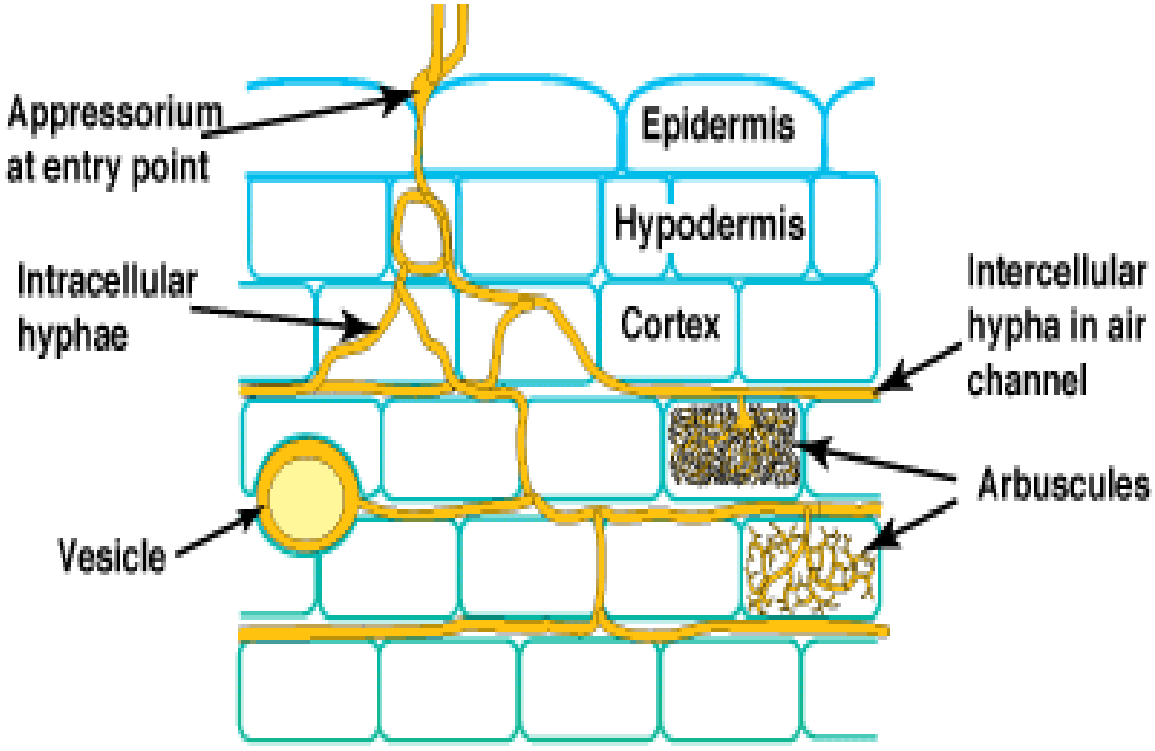

Fig.1.1: Schematic diagram of the characteristic structures of arbuscular mycorrhizal fungi.*

* (http://mycorrhizas.info/vam.html)

Outside the root, the external AM hyphae ramify extensively through the surrounding soil and acquire nutrients and water from the soil outside the root depletion zone. The external hyphae can extend several centimeters from the root surface and increase the root absorbing surface area, thus improving the ability of the plants to utilize soil resources more effectively (Li et al., 1991).

It is well known that the colonization of plant roots by AMF improves plant growth and productivity (Arias et al., 1991; Kleikamp and Joergensen 2006), mainly by increasing the uptake of nutrients, especially phosphorus (Bolan, 1991). AMF also increase the uptake of N (Mäder et al., 2000) and less mobile nutrients such as Zn, Cu, K and Ca (Marschner and Dell, 1994). Furthermore, AMF can protect host plants from root pathogens and water stress and contribute to soil aggregation and stability (Azcon-Aguilar and Barea, 1996; Auge, 2001; Rillig, 2004). However, the beneficial effect of mycorrhizae on plant growth is of particular significance in soils of low nutrient status (Arias et al., 1991; Azcón et al., 2003).

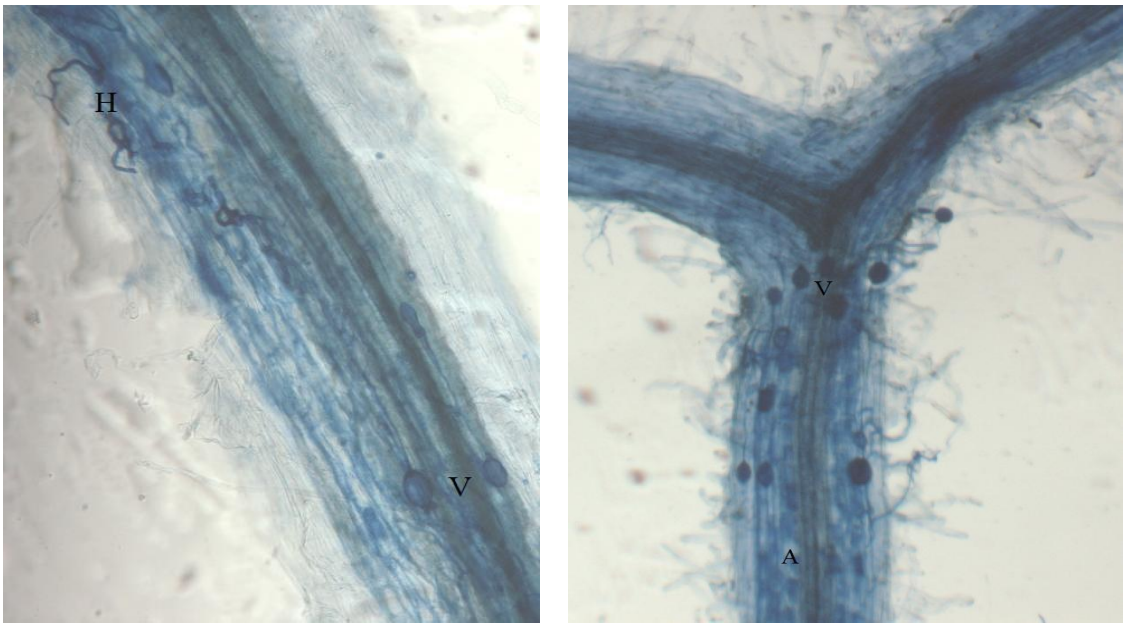

Fig.1.2: Microscopic images of pea roots colonized by arbuscular mycorrhizal fungi (AMF) at flowering stage. Roots were stained with trypan blue to show the distribution of fungal structures, vesicles (V), a: arbuscules (A), hyphae (H). Pot experiment 2010

Mycorrhizal colonization of plants especially of legumes plays an important role in plant growth by meeting the high phosphorus requirement for nodule formation and N_2 fixation (Arias et al., 1991; Stancheva et al., 2006). Peas have comparatively high mycorrhizal dependency and are considered to be the plant species that benefits most from mycorrhizal presence (Plenchette et al., 1983; Stancheva et al., 2006).

Colonization of pea roots by AMF may range from 4 to more than 90%, depending on land-use history and soil properties (Kleikamp and Joergensen, 2006).

There is substantial evidence that mycorrhizal fungi are capable of decomposing complex organic material by producing different extracellular enzymes. These findings apply especially to ericoid and ectomycorrhizal fungi but also to AMF (Dighton, 1991; Burke, 2011). AMF are obligate biotrophic and their ability to capture nutrients from organic sources directly remains a matter of debate. AMF have been found to penetrate throughout decomposing organic residues (Hodge, 2003). However, there is some evidence suggesting that AMF enhance residue decomposition (Hodge et. al., 2001; Tu et al., 2006; Leigh et al., 2009), by stimulating the activity of saprophytic microbes (Hodge et. al., 2001), or by producing extracellular enzymes (Koide and Kabir, 2000). The ^{13}C and ^{15}N-labeled C_4 plant material have been used successfully to study the decomposition process and to follow the distribution of the labeled material over different fractions in soil and plants (Zareitalabad et al., 2010; Rottmann et al., 2010).

1.3. Organic fertilizers

Organic agriculture is developing rapidly and is now practiced in more than 160 countries of the world (Willer and Kilcher, 2011). According to the latest survey on organic farming, about 37.2 million hectares are managed organically worldwide, which constitutes 0.9% of the agricultural land of the countries covered by the survey (Willer and Kilcher, 2011).

Organic farming depends mainly on the addition of organic fertilizers to soil (Herencia et al., 2008; Heinze et al., 2010). Incorporation of organic substrates into the soil is very important to maintain soil fertility and SOM and counteract nutrient depletion. Various kinds of organic materials such as animal manures, sewage sludge, and crop residues are applied to soil to improve soil organic matter content and, consequently, the physical, chemical and biological properties of the soil (Marinari et al., 2000; Heinze et al., 2010). Organic fertilizers provide the majority of essential plant nutrients, improving actual crop productivity, but also leaving beneficial residual effects on succeeding crops (Ghosh et al., 2004). However, less attention has been paid to some types of manure such as yard-waste compost from shrub and garden cuttings and especially fresh straw-rich horse manure that contain large amounts of woody debris and bedding straw, with a wide C:N ratio.

In recent years, biogenic municipal waste compost and yard-waste compost have been frequently used as soil amendments on agricultural land to improve soil fertility and crop production, with the additional benefit of reducing waste disposal costs (García-Gil et al., 2000; Pérez-Piqueres et al., 2006). The use of such waste composts may be an interesting and cheap substitute for manure, especially for farms with little or no livestock (Quintern et al., 2006). Yard-waste compost, which consists mainly of plant material and wooden debris, contains large amounts of lignin and lignin-cellulose complexes and acts as a long-term source of nutrients (Niklasch and Joergensen, 2001; Pérez-Piqueres et al., 2006).

Horse manure usually contains more bedding material compared to other animal manures (Airaksinen, 2006). Bedding material is used to improve the hygiene of the stables and to absorb urine, moisture and manure gases such as ammonia (Swinker et al., 1998; Airaksinen, 2006). Bedding material has a large effect on the quality of manure as fertilizer, and manures with high straw content can cause temporary N immobilization (Thomsen and Kjellerup, 1997), thus reducing soil N available for plant uptake. Cereal straws such as those from wheat and barley are the most frequently used bedding materials in horse stables (Werhahn et al., 2010).

Microbial decomposition of organic residues can be affected by several environmental factors (e.g. soil moisture content, soil temperature and O_2) but also by organic matter quality (Powlson et al., 2001). However, the decomposition process occurs within a complex environment which includes not only saprobic microorganisms, but also living roots and their symbiotic mycorrhizal fungi. The presence of growing plants may decrease (Muhammad et al. 2007) or increase (Paré et al., 2000) the decomposition of organic residues in soil.

Incorporation of organic fertilizers into soil causes a large and rapid increase in the soil microbial biomass (Rasul et al., 2008), which forms only a small fraction of soil organic matter (Anderson and Domsch, 1989), but plays an important role in nutrient cycling and plant nutrition, due to its fast turnover (Jenkinson and Ladd, 1981). For this reason, some studies have found a close relationship between the soil microbial biomass and crop yields under greenhouse conditions (Chen et al., 2000) as well as under field conditions (Khan and Joergensen, 2006; Mandal et al., 2007). However, this relationship was not always observed (Nillson et al., 2005).

1.4. Intercropping system

In recent times, there has been growing interest in diversified agricultural production systems to obtain improved crop protection, increased productivity and profitability offered by many intercropping system (Ghosh, 2004). Intercropping is the simultaneous cultivation of two or more crop species on the same area of land (Willey, 1979). Particularly, legume-cereal intercropping is widely practiced as a sustainable cropping system to enhance productivity and reduce the dependence on mineral N fertilizer. Intercropping of legumes with cereals is known to increase yields, yield stability and land use efficiency of intercrops compared to sole crops (Jensen, 1996; Hauggaard-Nielsen and Jensen, 2001). The advantages of intercropping have been attributed to the complementary use of growth resources such as light, water and nutrients by the component crops (Hauggaard-Nielsen et al., 2008). In addition, the legume component may contribute to the N-nutrition of an intercropped cereal via rhizodeposition, especially at low rates of N-fertilization (Jensen, 1996a). In an intercropping system, above and belowground interactions between species will affect the performance of N_2 fixation of the intercropped legume. Several studies have shown that the cereal component, which usually has a faster-developing and more extensive root system, is more competitive for soil N, forcing the legume crop to rely on N_2 fixation (Jensen, 1996; Andersen et al., 2004). Approximately, intercropped pea derived 84% of its N from biological fixation, whereas fixation contributed 62% of the N accumulated by sole pea (Izaurralde et al., 1992).

2. Objectivs:

As shown above, the challenge today is how to improve crop productivity while maintaining or even enhancing soil fertility. Organic fertilizer and biofertilizer such as mycorrhiza, legume-cereal intercrops, as well as microbial decomposition processes of organic material in soil, play essential roles in farming systems. This thesis represents interdisciplinary research at the interface between soil biology, decomposition processes, and crop science.

2.1. *Objectives of the pre- experiment: Influence of arbuscular mycorrhizal fungi on pea growth and the decomposition of maize straw. (Chapter 3)*

A pot experiment was conducted to study the effect of mycorrhizal infection on pea plant and the decomposition rate of maize straw, in which peas were grown in the soil with addition of NPK fertilizer and ^{15}N labeled maize leaf straw. Two pea *(Pisum sativum* L.) genotypes, the symbiotic mycorrhizal (*myc*$^{+}$) and nodulating (*nod*$^{+}$) parental isoline Frisson and its non-symbiotic non-mycorrhizal (*myc*$^{-}$) and non-nodulating (*nod*$^{-}$) isoline mutant P2 were grown in pots with and without ^{15}N labeled maize leaf straw. The aims of this experiment were: (1) to compare the yield between the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson and (2) to investigate the effects of AMF and the presence of living pea roots on the decomposition of maize straw.

In this experiment, mycorrhizal colonization was not observed. The fertilizer rate used was very high, so the experiment was replicated with the same procedures except the fertilizer rate, and is presented in Chapter 4.

2.2. *Objectives of the first experiment: Impact of pea growth and arbuscular mycorrhizal fungi on the decomposition of ^{15}N-labeled maize residues (Chapter 4).*

Two field pea (*Pisum sativum* L.) isolines, the symbiotic mycorrhizal (*myc*$^{+}$) and nodulating (*nod*$^{+}$) parental isoline Frisson and its non-symbiotic non-mycorrhizal (*myc*$^{-}$) and non-nodulating (*nod*$^{-}$) isoline mutant P2 were grown in pots with (*maize*$^{+}$) and without (*maize*$^{-}$) addition of ^{15}N-labelled maize residues (*Zea mays*) (Fig. 2.1) in order to (1) compare yield in C and N of different plant parts, nutrient concentrations and root

microbial colonization between the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson, (2) investigate the effects of AMF and growing pea plants (*myc*$^+$ and *myc*$^-$) on the decomposition and microbial use efficiency of ^{15}N-labeled maize residues, (3) characterize the distribution of the added substrate over different soil fractions, such as particulate organic matter, soil microbial biomass and microbial residues.

Fig.2.1: Pea plants 2 weeks after planting, from left to right: *Myc*$^+$ *maize*$^+$, *Myc*$^+$ *maize*$^-$, *Myc*$^-$ *maize*$^+$, *Myc*$^-$ *maize*$^-$

2.3. *Objectives of the second experiment: Organic fertilizer effects on pea yield, nutrient uptake, microbial root colonization and soil microbial biomass indices in organic farming systems (Chapter 5).*

In the field experiment, field peas (*Pisum sativum* L.) were grown as the sole crop or intercropped with oat (*Avena sativa* L.). The organic fertilizers used in the experiment were horse manure mixed with stall bedding and compost derived from shrub and garden cuttings, which were supplied at nearly equivalent N amounts but different C amounts (Fig. 2.2a,b). The objectives were (1) to evaluate the beneficial effects of C-rich organic fertilizers on pea productivity in different cropping systems and (2) to investigate whether these effects were reflected by microbial root colonization, microbial biomass and CO_2 production, and (3) to study the residual effects of the organic fertilizers on wheat as a succeeding crop in organic farming.

Fig.2.2a: Experimental plot fertilized with compost derived from shrub and garden cuttings, Frankenhausen, 2010

Fig.2.2b: Experimental plot fertilized with horse manure mixed with stall bedding, Frankenhausen, 2010.

2.4. *Objectives of the thrid experiment: Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions (Chapter 6).*

In a field experiment, peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) were grown as sole crops and intercrops (Fig. 2.3), fertilized with horse manure mixed with stall bedding and yard-waste compost derived from shrub and garden cuttings at 10 t C ha^{-1} each. The objectives were to compare the effects of this organic fertilizer and cropping system in organic farming on (a) yield of peas and oats, grown as the sole crop or intercropped, as well as N_2 fixation and photosynthetic rates, (b) the yield of wheat as a succeeding crop, (c) microbial biomass indices in soil and roots, and (d) microbial activity estimated by the CO_2 evolution rate in the field and the amount of organic fertilizers, recovered as particulate organic matter (POM).

Fig.2.3: pea intercropped with oat (a), sole pea (b), sole oat (c), Frankenhausen, 2010.

2.5. *Objective of the fourth study: Monitoring of crop biomass using vegetation indices derived from true color aerial photographs (Chapter 7).*

A remote-controlled hexacopter with an RGB digital camera was tested for evaluating the crop biomass. The hexacopter was flown over a field in which peas and oats were grown as sole crops and intercrops, fertilized with horse manure and yard-waste compost (10 t C ha^{-1}). The relationships between the Normalised Green-Red Difference Index (NGRDI) derived from aerial images and aboveground plant biomass, and LAI were tested. In addition, NGRDI data obtained from true color aerial images of the field were tested for spatial autocorrelation to predict spatial distribution of aboveground biomass within a managed field.

3. Preliminary experiment: Influence of arbuscular mycorrhizal fungi on pea growth and the decomposition of maize straw.

3.1. Introduction

Arbuscular mycorrhizal fungi (AMF) are the most intensively studied type of mycorrhizal association, because they are present in most agricultural and natural ecosystems (Marin, 2006). Mycorrhizal infection enhances plant growth and yield primarily by increasing nutrient uptake (Arias et al., 1991; Smith and Read, 2008), especially phosphorus (Bolan, 1991; Cavagnaro et al., 2006), by increasing the absorptive surface of root systems (Marschner and Dell, 1994), faster movement of P into hyphae and solubilization of soil phosphorus by release of organic acid and phosphatase enzymes (Bolan, 1991). However, this positive effect on plant growth is of particular importance in soils of low nutrient status (Liu et al., 2000). Although it is generally accepted that the level of root colonization by VAM fungi decreases with increased P availability (Liu et al., 2000; Breuillin et al., 2010), phosphorus fertilization does not necessarily reduce mycorrhizae to trace levels (Miller et al., 1995; Grant et al., 2005).

The influence of mycorrhizae on legumes is potentially greater than for other plant groups, because legumes have a high P requirement for nodule formation, nitrogen fixation and optimum growth (Hayman, 1986; Arias et al., 1991). Plants with coarse root systems with few short hairs, such as many legumes, show a high degree of mycorrhizal dependency than plants with extensive, fine root systems, such as grasses (Harinikumar and Bagyaraj, 1989). Geneva et al. (2006) demonstrated that dual inoculation of pea plants with AMF and *Rhizobium leguminosarum* significantly increased plant biomass, photosynthetic rate, nodulation, and nitrogen fixation activity compared to single inoculation of the host with *Rh. leguminosarum.* In white clover (*Trifolium repens* L.), mycorrhizal inoculation doubled P concentration in tissue of infected plants and increased their dry weight, irrespective of P levels (50 or 150 mg kg^{-1} soil) (Li et al., 1991).

AMF are thought to be unable to decompose organic material, due to the lack of saprotrophic capacity (Read and Perez-moreno, 2003). However, there is evidence that mycorrhizal fungi are capable of decomposing complex organic materials by producing different extracellular enzymes (Koide and Kabir, 2000). Similarly, other studies have shown that AMF can enhance residue decomposition by stimulating microbial activity, through enhanced labile C availability from the host plant (Hodge et al., 2001; Tu et al., 2006). However, there are some conflicting opinions regarding their ability to decompose organic matter.

The decomposition of crop residues and the resulting release of nutrients in plant-available forms are key functions of soil microorganisms (Swift et al., 1979). Plant residues are an important source of organic matter and are also known to improve the physical, chemical and biological properties of soils, thus maintaining soil fertility (Scheller and Joergensen, 2008). Microbial decomposition of organic matter depends mainly on soil temperature, soil moisture, and substrate quality. In addition, the presence of growing plants has been shown to affect residue decomposition in different ways (Dormaar, 1990). Plant roots release a wide range of organic compounds into the surrounding soil (Wichern et al., 2007). Root exudates and rhizodeposition are known to stimulate microbial growth and activity (Mayer et al., 2003). In contrast, plant roots may compete with soil microorganisms for nutrients and water, reducing microbial activity and turnover processes (Muhammad et al., 2007).

The aims of this study were (1) to compare yield and yield components between the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson; (2) to study the effect of maize straw addition on the content of microbial biomass and plant growth, and (3) to investigate the effects of AMF and the presence of living pea roots on the decomposition of maize straw.

3.2. Material and methods

3.3.1. Soil and plant material

The soil was taken in November 2008 at 0-15 cm depth from the site “Saurasen” in the north of Hessia, Germany, sieved (< 2 mm), homogenized, and stored in polyethylene buckets at room temperature for about 4 weeks before the experiment started. The soil developed from eroded loess overlying clayey sandstone and was classified as Stagnic Luvisol according to the WRB-FAO classification system

(Quintern et al., 2006). The soil contained 6% sand, 77% silt and 17% clay, with a pH (H_2O) of 6.5 and a water holding capacity of 50%. The soil contained 9.8 mg g^{-1} soil total C and 0.99 mg g^{-1} soil total N.

Two pea *(Pisum sativum* L.) genotypes, the symbiotic mycorrhizal (*myc*$^+$) and nodulating (*nod*$^+$) parental isoline Frisson and its non-symbiotic non-mycorrhizal (*myc*$^-$) and non-nodulating (*nod*$^-$) isoline mutant P2 (Duc et al., 1989) were used in this experiment. Seedlings were germinated on wet filter paper at 22 °C for four days.

3.3.2. Experiment design

The experiment was carried out in plastic pots, each filled with 2.5 kg soil (on an oven dry basis) on 2 December 2008. The experiment had six treatments, each replicated four times: (1) control soil without pea plants and without maize straw (*pea*$^-$ *maize*$^-$), (2) control soil without pea plants and with maize straw (*pea*$^-$ *maize*$^+$), (3) the non-symbiotic pea mutant P2 without maize straw (*myc*$^-$ *maize*$^-$), (4) the non-symbiotic pea mutant P2 with maize straw (*myc*$^-$ *maize*$^+$), (5) the symbiotic parental isoline Frisson without maize straw (*myc*$^+$ *maize*$^-$), (6) the symbiotic parental isoline Frisson with maize straw (*myc*$^+$ *maize*$^+$). In the respective treatments, maize leaf straw was mixed into the soil before filling into the pots. Maize straw contained 43.7% C and 2.5% N, with a $\delta^{13}C$ value of 13.89‰ and $\delta^{15}N$ value of 4660‰ (Zareitalabad et al., 2010), and was applied at the rate of 5 mg maize leaf straw g^{-1} soil, equivalent to 2.1 mg C and 125 µg N g^{-1} soil. In the pea treatments, three pre-germinated seeds of *myc*$^-$ and *myc*$^+$ peas were sown 7 days after the pot experiment started at a depth of 3 cm and thinned to two plants per pot after emergence. All 24 pots were inoculated with mycorrhizal fungi as granular preparation placed directly below the seed just prior to planting. All pots were fertilized with 375 mg N kg^{-1} soil as NH_4NO_3, 327 mg P and 411 mg K kg^{-1} soils as KH_2PO_4 at sowing. The pots were arranged in a randomized complete block design and placed in a greenhouse. During the experiment, the moisture was maintained at 50% of the water-holding capacity by weighing and adding the water lost regularly twice a week.

3.3.3. Soil and plant sampling

Soil samples of 60 g fresh weight were taken from the pots at day 0 and at day 7 (before sowing) for the determination of microbial biomass in soil. The numbers of

flowers were recorded every two days (Fig. 3.1). At maturity (20 March 2009; 101 days after sowing), aboveground plant biomass was harvested by cutting the plant stem at the soil surface. Plant height was measured from the base of the plant to the tip of the central axis. The number of pods and seeds per plant were counted. The root system of each plant was then gently separated from soil and washed thoroughly with water. Fresh root samples of 1.5 g were taken for measuring mycorrhizal colonization and the other root material was dried. The soil in each pot was thoroughly mixed and sieved through a 2 mm mesh sieve. Pea roots and maize straw > 2 mm were separated with tweezers. This maize straw was the coarse (> 2 mm) particulate organic matter (POM) fraction. A sub-sample of this soil (400 g) was used for determination of the fine (0.4-2 mm) POM fraction. The remaining soil was stored in plastic bags at 4°C until the biological analyses started. Pea grains were air dried while shoots (included all remaining parts of plant except the grains) and roots biomasses were oven dried at 60°C to constant weight.

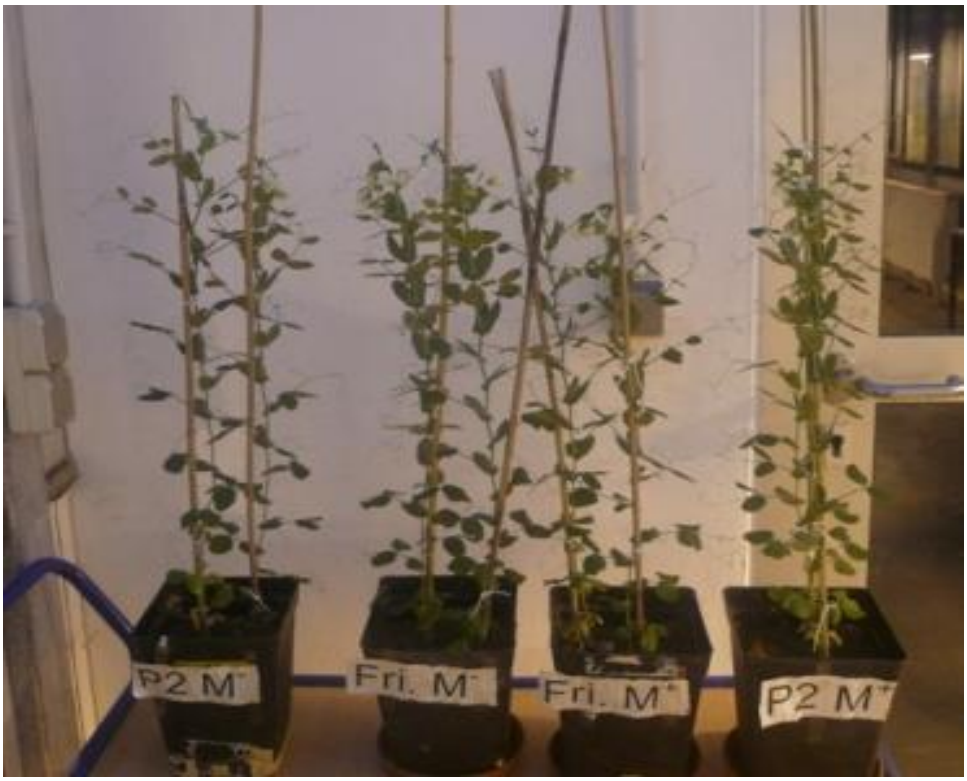

Fig.3.1: Pea plants at flowering stage, from left to right: *Myc*$^{-}$ *maize*$^{-}$, *Myc*$^{+}$ *maize*$^{-}$, *Myc*$^{+}$ *maize*$^{+}$, *Myc*$^{-}$ *maize*$^{+}$.

3.3.4. Microbial biomass C, N, and P

Microbial biomass C and N were estimated using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Moist soil (of 20 g was split into two portions of 10 g. One portion was fumigated for 24 h with ethanol-free $CHCl_3$. After its removal, the soil was extracted with 40 ml of 0.05 M K_2SO_4 (Potthoff et al., 2003) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The non-fumigated portion was extracted in the same way. Organic C in the K_2SO_4 extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was E_C/k_{EC}, where E_C = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion using a Dimatoc 100 automatic analyzer. Microbial biomass N was E_N/k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985).

Soil microbial biomass P was measured by fumigation extraction (Brookes et al., 1982). Three portions equivalent to 2.5 g oven-dry soil were each extracted with 50 ml of 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 150 rev min^{-1}, centrifuged for 10 minutes at 2000xg and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The first portion was fumigated (see above), the second portion was non-fumigated and the third portion was used for estimating the recovery of 25 μg P g^{-1} soil added to the extractant as KH_2PO_4. Phosphorus was analyzed by an ammonium molybdate-ascorbic acid method as described by Joergensen et al. (1995). Microbial biomass P was E_P / k_{EP} / recovery, where E_P = (PO_4-P extracted from fumigated soil) - (PO_4-P extracted from non-fumigated soil) and k_{EP} = 0.40 (Brookes et al. 1982).

3.3.5. Mycorrhiza colonization

Fresh root material of 1.5 g was cut into 1 cm lengths, mixed thoroughly, and then cleared by boiling in 10% KOH. After rinsing with tap water, the samples were boiled for 3 min in a 5 % ink-acetic acid solution, (Blue ink, Pelikan, Hanover, Germany) (Vierheilig et al., 1998). Subsequently the roots were destained by rinsing in tap water acidified with a few drops of 5% acetic acid, and stored in tap water until examination. The percentage of root length colonized was determined with the gridline intersection

method (Giovannetti and Mosse, 1980), using a binocular microscope at × 40 magnification.

3.3.6. Particulate organic matter (POM)

Particulate organic matter was determined according to Magid and Kjaergaard (2001) as described by Muhammad et al. (2006). Moist soil of 400 g was dispersed in 500 ml of 5% NaCl, shaken by hand and allowed to stand for 45 min. Then, the samples were poured gradually onto a sieve of 400 μm mesh size and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve until the water passing through the sieve became clear. The material retained on the sieve was transferred into a bucket. Tap water was added, the bucket was swirled and organic material was separated repeatedly from the mineral material by flotation-decantation, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining particulate organic matter POM > 400 μm was transferred to a crucible, dried at 60 °C, weighed and ground for analysis.

3.3.7. Calculation and statistical analysis

Aliquots of plant material (shoot and root) and the two POM fractions (0.4-2 and >2 mm) were burned in a muffle furnace to ash at 550 °C for 10 hours, and reweighed to calculate the ash-free dry mass. Organic C (%) was calculated as follows (Armecin et al., 2008):

$$\%MM = \frac{AW}{DW} \times 100 \quad (1)$$

$$\%OM = \frac{DW - AW}{DW} \times 100 \quad (2)$$

$$\%OC = \frac{\%OM}{1.724} \quad (3)$$

where MM = mineral matter or ash, AW = ash weight of the sample, DW = dry weight of the sample, OM = organic matter, OC = organic carbon and 1.724 = conversion factor (i.e. organic matter contains 58% OC).

Assuming that the addition of maize leaf straw did not affect the decomposition of native soil organic matter, the percentage of maize-C decomposed (MD) was calculated as follows (Muhammad et al., 2007a):

$$MD = \left[\frac{POM - C_{maize^+} - POM - C_{maize^-}}{C_{Added}} \right] \times 100 \quad (4)$$

Where POM-C_{maize}^{+} and POM-C_{maize}^{-} represent average POM-C recovered in the two fractions, 0.4-2 and >2mm in the *maize*$^{+}$ and the respective *maize*$^{-}$ treatments, respectively. C_{added} represents the amount of initially added organic C with maize.

Statistical analyses were carried out using SPSS statistical software (SPSS 15.0). The significance of experimental effects was tested by a two-way ANOVA in tables, and by a one-way ANOVA in figures using the LSD test ($P<0.05$).

3.3. Results

3.3.1. Mycorrhizal colonization and plant response

Flowering time differed slightly between the *myc*$^{+}$ and *myc*$^{-}$ plants (Fig. 3.2). The *myc*$^{-}$ plants began to flower at 48 day after sowing (DAS), whereas *myc*$^{+}$ plants flowered 3-6 days later. The peak of inflorescence of *myc*$^{+}$ plants occurred also a few days later. The root samples for mycorrhizal assessment were taken at the maturity stage (101 DAS). The microscopic examination showed that neither *myc*$^{+}$ plant roots nor mutant myc^{-} roots were colonized by mycorrhizal fungi.

In general, the differences between *myc*$^{-}$ and the parental *myc*$^{+}$ plants were not large. Plant height ranged from 73 to 80 cm without any treatment effect (Table 3.1). The number of pods and grains per plant were slightly higher in the *myc*$^{+}$ than in the *myc*$^{-}$ plants. Grain and root yields of the *myc*$^{+}$ plants were higher by approximately 17 and 85 %, respectively, than those of *myc*$^{-}$ plants. Shoot yield did not differ significantly between the two pea isolines, consequently, the shoot to root ratio of *myc*$^{+}$ plants was significantly lower than that of *myc*$^{-}$ plants (Table. 3.2). The presence of maize leaf straw increased only root yield of both isolines. The amount of root C of the *myc*$^{+}$ plants was also higher by 65%. The *myc*$^{+}$ plants had also a higher amount of root C and lower shoot-C to root-C ratio (Table. 3.3).

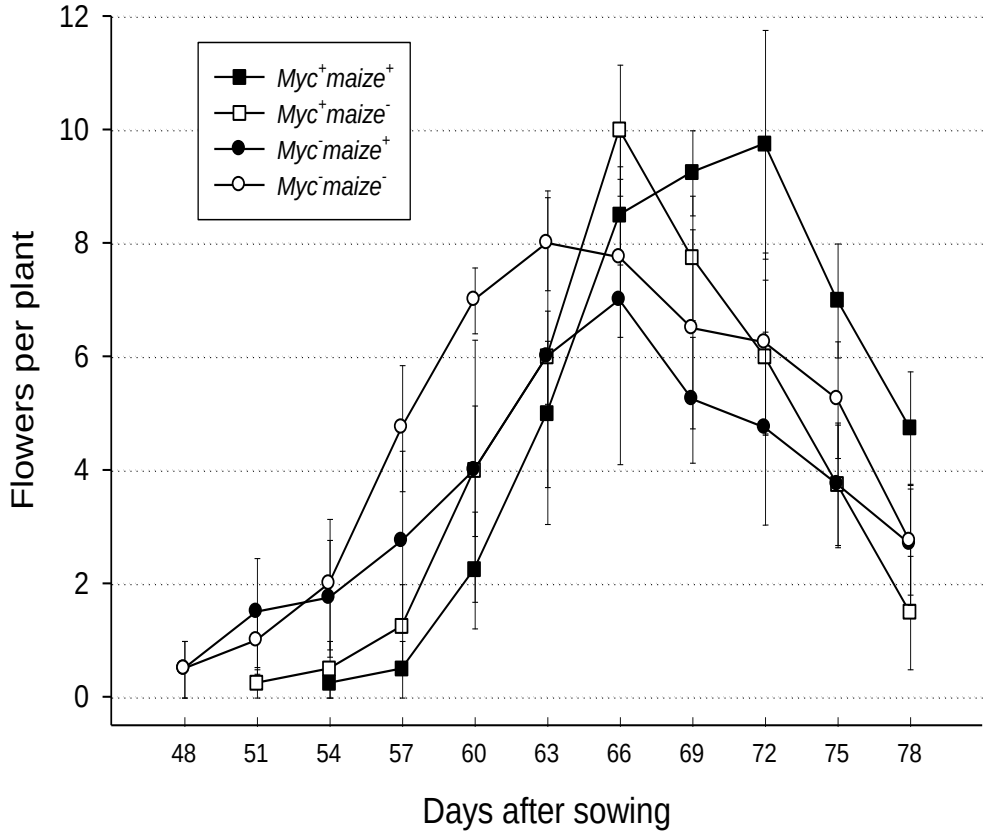

Fig.3.2: Flowering of *myc*$^{+}$ and *myc*$^{-}$ peas, grown in soil without (*maize*$^{-}$) and with (*maize*$^{+}$) maize straw; bars = ±1 standard error of mean (n = 4).

Table 3.1: Plant height, pod and grain yields of *myc*$^{+}$ and *myc*$^{-}$ peas, grown in soil without (*maize*$^{-}$) and with (*maize*$^{+}$) maize straw, at 101 days after sowing.

Treatments	Plant height (cm)	Pods (n plant^{-1})	Grain (n plant^{-1})
Myc^{-} *maize*$^{-}$	73	8.3	31
Myc^{-} *maize*$^{+}$	79	7.1	32
Myc^{+} *maize*$^{-}$	76	8.1	35
Myc^{+} *maize*$^{+}$	80	10.6	38
Probability values			
Myc	NS	0.04	0.07
Maize	NS	NS	NS
Myc × *maize*	NS	NS	NS
CV (± %)	7	18	12

CV= mean coefficient of variation between replicate pots (n = 4); NS = not significant.

Table 3.2: Grain, shoot and root dry matter yields as well as shoot to root ratio of *myc*$^+$ and *myc*$^-$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize straw at 101 days after sowing.

Treatments	Grain*	Shoot[1]	Root	Shoot / Root
	(g pot^{-1})			
Myc$^-$ *maize*$^-$	9.7	7.2	0.81	8.9
Myc$^-$ *maize*$^+$	9.9	8.0	1.15	6.8
Myc$^+$ *maize*$^-$	11.4	8.4	1.35	6.4
Myc$^+$ *maize*$^+$	11.5	8.7	2.30	3.8
Probability values				
Myc	0.06	0.08	<0.01	<0.01
Maize	NS	NS	0.01	0.01
Myc × *maize*	NS	NS	NS	NS
CV (± %)	12	12	24	26

CV= mean coefficient of variation between replicate pots (n = 4); NS = not significant; * = air-dried; [1]shoot biomass excluding grains.

Table 3.3: Shoot-C and root-C yields as well as Shoot-C to root-C ratio of *myc*$^+$ and *myc*$^-$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize straw, at the end of the 101-day pot experiment.

Treatments	Shoot-C	Root-C	Shoot-C / Root-C
	(g pot^{-1})		
Myc$^-$ *maize*$^-$	3.6	0.33	10.9
Myc$^-$ *maize*$^+$	3.9	0.44	9.0
Myc$^+$ *maize*$^-$	4.1	0.55	7.9
Myc$^+$ *maize*$^+$	4.3	0.71	6.2
Probability values			
Myc	NS	<0.01	0.02
Maize	NS	NS	NS
Myc × *maize*	NS	NS	NS
CV (± %)	23	23	24

CV= mean coefficient of variation between replicate pots (n = 4); NS = not significant

3.3.2. Microbial biomass and particulate organic matter (POM)

At day 0, i.e. immediately after the addition of maize straw, the contents of microbial biomass C, biomass N and biomass P increased significantly by 43%, 60%, and 24%, respectively (Table 3.4). At day 7, i.e. immediately before sowing of pea plants, these three microbial indices remained nearly constant in the *maize*$^-$ treatment. In the *maize*$^+$ treatment, only microbial C and N were further increased up to 650 and 90 µg g^{-1} soil, respectively. It is important to note that at day 0 and day 7 the microbial biomass was extracted without removing the visible particles of maize straw from the soil. At the end of the experiment, neither the growth of pea plants nor the addition of maize straw alone led to a significant increase in microbial biomass C (Fig. 3.3). In contrast, the combination of growing peas and maize straw significantly increased microbial biomass C by 55% in the *myc*$^+$*maize*$^+$ and by 28% in the *myc*$^-$ *maize*$^+$ in comparison with the treatment *pea*$^-$ *maize*$^-$. The amounts of maize-derived C recovered in the two POM fractions 0.4 – 2 and > 2 mm were significantly higher in the presence of pea plants in comparison with unplanted soil (Table 3.5). On the basis of the difference between straw-C added and straw-C recovered (Eq.4), 92% of the straw added was decomposed in unplanted soil and 87% in the presence of *pea* plants (84% in Frisson and 89% in P2)

Table 3.4: contents of microbial biomass C, N and P in the soil without (*maize*$^-$) and with (*maize*$^+$) maize straw at day 0, immediately after maize addition, at day 7 immediately before sowing of peas

Sampling day	Treatment	Microbial biomass (µg g^{-1} soil)		
		C	N	P
Day 0	*Maize*$^-$	345	45	21
	Maize$^+$	490	72	26
Day 7	*Maize*$^-$	280	46	15
	Maize$^+$	650	89	22
Probability values				
Sampling day		NS	0.07	0.04
Maize		<0.01	<0.01	0.03
Sampling day × *maize*		<0.01	NS	NS
CV (± %)		17	14	23

CV= mean coefficient of variation between replicate measurements (n = 4 at day 0, n = 12 at day 7.

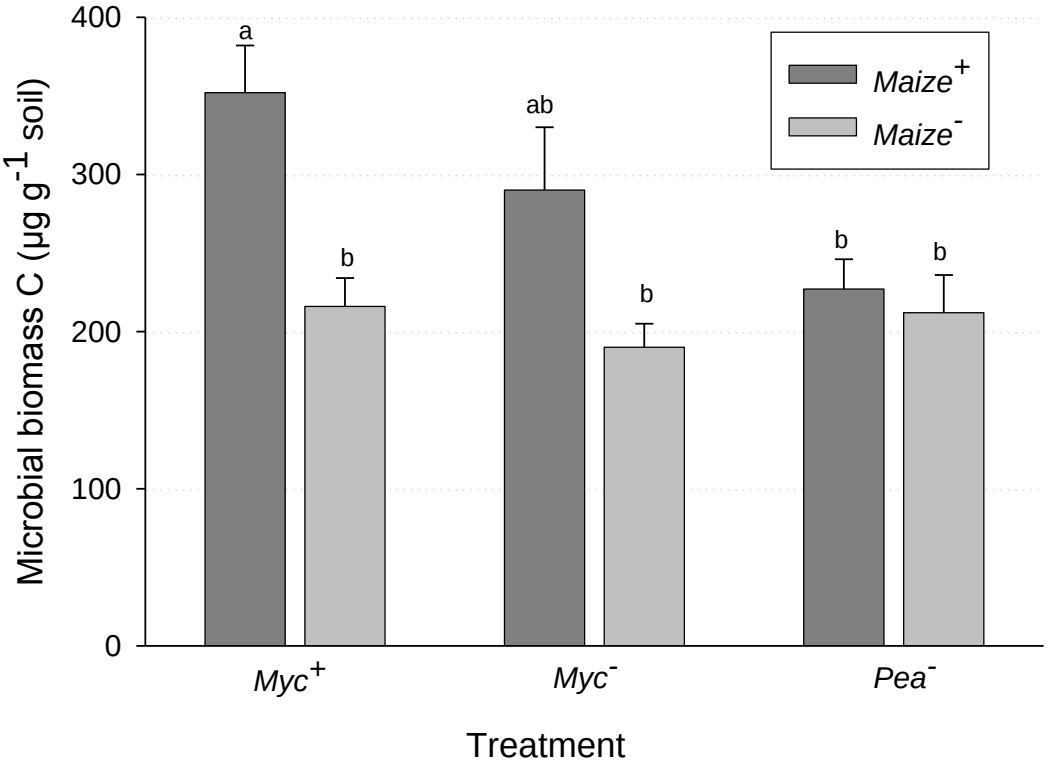

Fig.3.3: Contents of microbial biomass C in pots with *myc*$^+$ and *myc*$^-$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize straw, in pots without peas (*pea*$^-$), without (*maize*$^-$) and with (*maize*$^+$) maize straw at the end of the experiment; vertical bars show ± one standard error (n = 4); different letters above the bars indicate a significant difference according to the LSD test (P<0.05).

3.4. Discussion

3.4.1. Plant responses

At the end of the experiment, no mycorrhizal infection was observed in the *myc*$^+$ roots. It has been shown that application of high amounts of phosphorus fertilizer suppresses the colonization of roots by mycorrhizal fungi (Breuillin et al., 2010; Balzergue et al., 2011). Amijee et al. (1989) reported that the rate of mycorrhizal infection decreased when the soil level of bicarbonate-soluble phosphorus exceeded 140 mg kg^{-1}. High P levels in soil have been shown to reduce the root membrane permeability and consequently the root exudates (Graham et al., 1981; Mohammed et al., 1998; Grant et al., 2005), which play a key role in the stimulation of the growth of mycorrhizal fungi (Tawaraya et al., 1998).

Table 3.5: Organic C content in the two particulate organic matter (POM) fractions 0.4 - 2 mm and >2 mm recovered from pots with *myc*$^+$ and *myc*$^-$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize straw, from pots without peas (*pea*$^-$), without (*maize*$^-$) and with (*maize*$^+$) maize straw at the end of the experiment.

Treatment	POM-C (μg g^{-1} soil)	
	(>2 mm)	(0.4 - 2 mm)
Pea$^-$ *maize*$^-$		31
Pea$^-$ *maize*$^+$	50	160
Myc$^-$ *maize*$^-$		78
Myc$^-$ *maize*$^+$	72	228
Myc$^+$ *maize*$^-$		94
Myc$^+$ *maize*$^+$	93	310
Porbability values *pea*$^-$ / *pea*$^+$		
Peas	0.03	0.02
Maize		< 0.01
Peas × *maize*		NS
Porbability values *myc*$^-$ / *myc*$^+$		
Myc	NS	NS
Maize		<0.01
Myc × *maize*		NS
CV (± %)	25	23

CV= mean coefficient of variation between replicate measurements (n = 4)

In addition, high P availability in soil reduces mycorrhizal development by reducing strigolactone production in roots (Yoneyama et al., 2007). Strigolactones are important secondary metabolites in plants and are required by AMF for rapid colonization of their hosts (Steinkellner et al., 2007). However, Lu et al. (1994) observed a considerable colonization of roots growing outside the P-treated zone, regardless of the very high soil P concentration in the P-treated zone. The *myc*$^-$ mutant P2 plants were not suffering from P deficiency, as indicated by the same flowering patterns of *myc*$^+$ and *myc*$^-$ plants. In contrast, Kleikamp and Joergensen (2006) observed a different inflorescence behavior of mutant P2, with a considerably earlier flowering peak than that of *myc*$^+$ Frisson. In this experiment, it was necessary to evaluate the level of mycorrhizal colonization at earlier stages of plant growth, e.g. flowering, since the level of colonization has been reported to decline during maturation (Mohammad et al., 1998;

Vestberg et al., 2010), due to the decrease in photosynthetic rate and translocation of nutrients from the leaves to the developing seeds, which reduces the photosynthate supply to the roots (Mohammed et al., 1998).

No significant differences in plant yields between the *myc*$^{+}$ and *myc*$^{-}$ plants were observed, similarly to the results reported by Kleikamp and Joergensen (2006). In their experiment, using P-deficient soil and two levels of P fertilization, the mutant P2 plants achieved the same grain yield as the parental symbiotic Frisson. Legumes generally do not require nitrogen fertilizer because of their ability to fix atmospheric nitrogen. In this study, the addition of high amounts of fertilizer N (375 mg kg^{-1} soil) at sowing may have strongly inhibited nodulation and N_2 fixation in Frisson plants (Peoples et al., 1995). However, traces of nodules were present on Frisson roots at harvest, although they were scarce.

The *myc*$^{-}$ mutant P2 had a lower root weight and consequently a larger shoot–to–root ratio than Frisson. AMF are known to promote root growth (Jackson et al. 2002) through the allocation of photosynthate C to the roots (Miransari et al., 2008). However, further genetic defects cannot be completely excluded, which would limit the utilization of such mutant plants for the evaluation of AM symbiosis (Kleikamp and Joergensen, 2006).

As is known, soil organic matter enhances soil fertility and improves production by improving the ability of the soil to store and supply nutrients. In this experiment, the addition of maize straw had no significant effect on yield indices. Clearly pea plants were able to utilize the high levels of added fertilizer without the need for further nutrient source. Therefore, the influence of maize straw addition as organic matter on plants was not noticed. In contrast, Muhammad et al. (2007) discovered that the dry weight of the maize shoot material was more than doubled in the treatment with alfalfa residues (C/N ratio of 12.5) than without. They assumed that this increase in maize growth after the addition of alfalfa was mainly due to the release of nutrients during decomposition.

3.4.2. Microbial decomposition

The addition of easily decomposable green maize leaves (C: N ratio, 17) increased significantly the contents of microbial biomass C, N and P at day 0 and 7. It has been repeatedly shown that the application of organic matter in the form of straw to the soil stimulates soil microbial activity, leading to an increase in microbial biomass

(Rottmann et al., 2011; Scheller and Joergensen, 2008). The increase of microbial biomass at day 0 immediately after incorporation of the straw into the soil may be due to the strong microbial colonization of the added straw (Potthoff, 2001; Muhammed et al., 2006). At the end of the experiment, only the combination of growing pea plants and maize straw significantly increased the content of microbial biomass C, similar to the findings of Muhammed et al. (2007a). Plant growth stimulates microbial growth and activity in the rhizosphere via rhizodeposition (Joergensen, 2000; Mayer et al., 2003). However, the effect of pea growing alone on the microbial biomass C was negligible.

At the end of the experiment, only 8 % of the maize straw added in the *pea*$^-$ and on average 13 % of that in the presence of *pea* plants were recovered as total POM, indicating nearly complete decomposition. However, the POM recovery in the *myc*$^+$ was 15%, compared with 10 % in the *myc*$^-$. The reasons for this rapid decomposition may be the low C/N ratio of the added green maize leaf straw and N availability to soil microorganisms (Rottmann et al., 2011). It is worth noting that the decomposition rate of added maize straw was significantly reduced by the presence of living roots. Jingguo and Bakken (1997) reported that competition between plants and microbes for available N may have suppressed microbial activity, thereby inhibiting organic matter decomposition. Muhammed et al. (2007) reported that water deficiency of soil microorganisms in the increased presence of plants roots and the shortage of nutrients such as phosphorus and sulfur that are needed for microbial decomposition might be the reasons for the retardation in the decomposition of alfalfa residues in the presence of maize plants. Other possible factors may be considered as an explanation for this observation: (1) preferential utilization by soil microorganisms of fresh materials (rhizodeposition) released from roots rather than the added organic residues (Nicolardot et al., 1995; Dormaar, 1990); (2) inhibition of the microbial activities by material produced by the roots or rhizosphere microbes (Reid and Goss, 1982). However, our results contrast the findings of Paré et al. (2000), who reported that the presence of growing plants increased alfalfa residue N mineralization.

3.5. Conclusions

The fertilizer rate used in this experiment was relatively high. No mycorrhizal infection was found in Frisson roots. High fertilizer application inhibited mycorrhizal development. Plant biomass and grain production did not differ between the two pea

isolines, with the exception of root weight, which was significantly higher in Frisson. The lack of biological N_2 fixation in mutant P2 did not limit growth and yield. The incorporation of maize straw stimulates soil microbial biomass, but not the plant biomass. The recovery of non-decomposed straw by the sieving procedure indicated nearly complete decomposition of maize straw, both in the presence and absence of pea plants, but the decomposition was significantly retarded by the presence of living pea roots.

4. Impact of pea growth and arbuscular mycorrhizal fungi on the decomposition of ^{15}N-labeled maize residues

Biology and fertility of soil (2011)

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Abstract

A pot experiment was carried out (1) to compare C and N yield of different plant parts, nutrient concentrations and root colonization between the non-mycorrhizal mutant P2 (*myc*$^-$) and the symbiotic isoline Frisson (*myc*$^+$), (2) to investigate the effects of AMF and growing pea plants on microbial decomposition of ^{15}N-labeled maize residues, and (3) to follow the distribution of the added substrate over different soil fractions, such as particulate organic matter, soil microbial biomass and microbial residues. Yields of C in straw, grain and roots of *myc*$^+$ peas were significantly higher by 27, 11 and 92%, respectively, compared with those of *myc*$^-$ peas. The δ^{13}C values in the different plant parts were significantly higher in *myc*$^+$ than in *myc*$^-$ tissue with and without maize. Application of labeled maize residues generally resulted in ^{15}N enrichment of pea plants. At the end of the experiment, the ergosterol concentration in roots of mature peas did not differ between the two isolines, indicating similar colonization by saprotrophic fungi. The decomposition of added maize residues was significantly reduced by the *myc*$^-$ peas, but especially by *myc*$^+$ peas. The formation of microbial residue C was increased and that of microbial residue N was reduced in the presence of plants. The insufficient N supply to soil microorganisms reduced decomposition of maize residues in the presence of peas, especially *myc*$^+$ peas.

Keywords: arbuscular mycorrhizal fungi, peas, maize residues, decomposition, microbial biomass, particulate organic matter, $\delta^{13}C$, $\delta^{15}N$

4.1. Introduction

Microbial decomposition of plant residues in soil is a central process for the release of nutrients and thus for maintaining nutrient cycling, and it can be affected by plants in different ways (Haider et al. 1989; Paré et al. 2000). Plants lower the water content by transpiration, reducing microbial activity and turnover processes (Franzluebbers et al. 1995; Reth et al. 2005). Plant roots change the pH by the release of H^+ or HCO_3^- ions, which may decrease or increase microbial turnover processes. Plant roots release rhizodeposits, increasing microbial growth and turnover processes (Wichern et al. 2007; Fustec et al. 2010). Plant roots create a rhizosphere-specific microbial community (Costa et al. 2006), altering the saprotrophic capability in unknown directions. Plants may compete with soil microorganisms for nutrients, especially in the rhizosphere (Schimel et al. 1989; Jingguo and Bakken 1997). These different directions of plant effects may be the reason for conflicting plant growth effects on microbial decomposition of plant residues (Dormaar 1990), leading to negative (conserving) effects (Nicolardot et al. 1995; Muhammad et al. 2006) or positive (priming) effects (Paré et al. 2000; Dijkstra et al. 2010).

In agricultural ecosystems, a specific feature of most crop and grassland species, especially of legumes, is the presence of arbuscular mycorrhizal fungi (AMF), which transfer between 5 to 20% of plant assimilates into the soil (Jones et al. 2004; Fitter et al. 2011). It is well known that AMF can improve nutrient acquisition and yield of host plants (Smith and Read 2008), especially that of P and N (Maeder et al. 2000, Cavagnaro et al. 2006). However, this positive AMF effect on plant decreases under conditions of non-limiting nutrient availability, especially P, after fertilizer application (Thingstrup et al. 1998; Kleikamp and Joergensen 2006). The presence of AMF may have stimulatory or inhibitory effects on soil microorganisms by several mechanisms (Christensen and Jakobsen 1993; Wamberg et al. 2003; Vestergård et al. 2008), including (1) quantitative and qualitative changes in root exudation, (2) competition for nutrients and water, and (3) exudation of stimulatory or inhibitory substances. However, AMF do not always have effects on microbial growth and activity (Olsson et al. 1996;

Cavagnaro et al. 2006). In addition, the role of AMF in the decomposition of organic amendments is not fully clear. As AMF are obligate biotrophic, it is generally thought that they are unable to decompose organic residues (Read and Perez-Moreno 2003). However, there is some experimental evidence for the role of AMF in decomposition processes in soil (Hodge et al. 2001). For example, Leigh et al. (2009) showed for *Glomus intraradices* and *G. hoi* that AMF can acquire up to one-third of the ^{15}N added as organic source.

Non-mycorrhizal mutants can be an important tool for studies on the interactions between AMF and other rhizosphere microorganisms without the demand for soil sterilization (Endlweber and Scheu 2006; Vestergård et al. 2008) or pasteurisation (Endlweber and Scheu 2006), which have strong impacts on soil chemistry and soil biology, by mobilizing nutrients such as N and P (Kahiluoto et al. 2000; Endlweber and Scheu 2006) and by killing many other soil organisms (Khan et al. 2010). The *myc*$^-$ mutants as non-mycorrhizal controls are available for tomatoes (Canavagro et al. 2006) but also for peas (Duc et al. 1989; Kleikamp and Joergensen 2006). Peas (*Pisum sativum* L.) are an important component of arable crop rotations in organic farming systems and the bibliography about their rhizodeposition is extensive (Mayer et al. 2003; Wichern et al. 2007).

The use of ^{13}C and ^{15}N labeled C_4 plant material makes it possible to distinguish between the microbial use of plant-derived C and that of soil organic matter derived from C_3 plants (Butenschoen et al. 2007). Consequently, it is possible to follow the distribution of the maize residues on different fractions, such as particulate organic matter, microbial biomass and microbial residues (Zareitalabad et al. 2010). The isotopic signature of different plant organs gives additional information on the effects of AMF on the physiological reaction of the host plant (Querejeta et al. 2003). The presence of AMF affects $\delta^{15}N$ (Handley et al. 1993; Wheeler et al 2000) as well as $\delta^{13}C$ values (Handley et al. 1993; Querejeta et al. 2003) differently in different plant parts, due to generic differences between autotrophic and heterotrophic organs (Yoneyama et al. 2003; Cernusak et al. 2009). AMF have been reported to decrease $\delta^{15}N$ in legumes, because mycorrhizal plants take up N from sources that are not or are less available to non-mycorrhizal plants (Ames et al. 1984; Ibijbijen et al. 1996). The $\delta^{13}C$ values in plant organic matter reflect net photosynthesis and water use efficiency in C_3 plants (Farquhar et al. 1989). Mycorrhizal plants were found to have higher water use

efficiency and consequently higher $\delta^{13}C$ values than non mycorrhizal ones (Querejeta et al. 2003).

The present pot experiment is based on the following hypotheses: (1) under conditions of non-limiting nutrients, the presence of AMF in *myc*$^+$ roots (a) has only small effects on plant growth, (b) but increases $\delta^{13}C$ values and decreases $\delta^{15}N$, and (c) reduces root colonization by saprotrophic fungi. (2) The presence of AMF further reduces the decomposition of maize residues by plant growth. (3) This reduction increases the substrate use efficiency of soil microorganisms, i.e. a higher percentage of the decomposed substrate is converted into microbial biomass C and N. The specific aims of the present pot experiment were to compare yield in C and N of different plant parts, nutrient concentrations and root microbial colonization between the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson, to investigate the effects of AMF and growing pea plants (*myc*$^+$ and *myc*$^-$) on the decomposition and microbial use efficiency of ^{15}N-labeled maize residues.

4.2. Materials and methods

4.2.1. Soil and maize residues

The soil was taken in September 2009 at 0-15 cm depth from the site "Saurasen" in the north of Hessia, Germany, sieved (< 2 mm), homogenized, and stored in polyethylene buckets at room temperature for about 6 weeks before the experiment started. The soil with 6% sand, 72% silt and 22% clay has been developed from eroded loess overlying clayey sandstone and was classified as Stagnic Luvisol according to the WRB-FAO classification system (Quintern et al. 2006). The soil contained 8.23 mg g^{-1} soil organic C and 0.88 mg g^{-1} soil total N with a pH ($CaCl_2$) of 6.4 and a water holding capacity of 50%. The $\delta^{13}C$ and $\delta^{15}N$ values of the bulk soil were -26.9 ± 0.2 ‰ and +6.4 ± 0.25 ‰, respectively.

The ^{15}N-labelled maize (*Zea mays* L.) residues were obtained in a greenhouse using ^{15}N-labelled ammonium nitrate solution. Maize was harvested six months after planting. After air drying, maize leaf residues were chopped into pieces of approximately 4 mm. Aliquots of the maize residues were dried at 60°C and ball-milled prior to isotopic analysis. Maize residues contained 42.22% C and 1.78% N, with a $\delta^{13}C$ value of -12.16‰ and a $\delta^{15}N$ value of 595‰.

4.2.2. Pot experiment

The experiment was carried out in plastic pots, each filled with 2.4 kg soil (on an oven-dry basis) on 2 November 2009. The experiment had six treatments, each replicated four times: (1) control soil without pea plants and without maize residues (*pea*$^-$ *maize*$^-$), (2) control soil without pea plants and with maize residues (*pea*$^-$ *maize*$^+$), (3) the non-symbiotic pea mutant P2 without maize residues (*myc*$^-$ *maize*$^-$), (4) the non-symbiotic pea mutant P2 with maize residues (*myc*$^-$ *maize*$^+$), (5) the symbiotic parental isoline Frisson without maize residues (*myc*$^+$ *maize*$^-$), (6) the symbiotic parental isoline Frisson with maize residues (*myc*$^+$ *maize*$^+$). In the respective treatments, maize residues were thoroughly mixed into the soil before filling into the pots. The application rate was 2.5 g maize residues kg^{-1} soil, equivalent to 1.05 mg C g^{-1} soil and 44.5 µg N g^{-1} soil. The pots were covered and incubated.

The symbiotic mycorrhizal (*myc*$^+$) and nodulating (*nod*$^+$) parental isoline Frisson and its non-symbiotic non-mycorrhizal (*myc*$^-$) and non-nodulating (*nod*$^-$) isoline mutant P2 (Duc et al. 1989) were used in the pea (*Pisum sativum* L.) treatments. Seeds were soaked in distilled water for 10 min, followed by immersion in ethanol (96%) for 5 min. After thorough washing with distilled water, seeds were immersed in a 10% H_2O_2 solution for 5 min and then rinsed again with distilled water and germinated at 22°C in the light for 4 days on moist filter paper. Three pre-germinated seeds of *myc*$^-$ and *myc*$^+$ peas were sown 9 days after the pot experiment started at a depth of 3 cm and thinned to two plants per pot after emergence. All 24 pots were inoculated with mycorrhizal fungi placed directly below the seed just prior to planting. The inoculum was added as (1) 0.75 g fresh mass of chopped *Tagetes sp*. roots, colonized by *Glomus etunicatum* (Becker & Gerdemann), *Glomus intraradices* (Schenck & Smith) and *Glomus claroideum* (Schenck & Smith) (INOQ, Schnega, Germany), together with (2) 8 g of dry granules, which contained attapulgite clay colonized by mycelium and spores of mainly *Glomus mosseae* (Rootgrow Professional, Kent, UK). All pots were fertilized with 20 µg N g^{-1} soil as NH_4NO_3, 40 µg P and 50 µg K g^{-1} soils as KH_2PO_4 at sowing, and 30 µg N g^{-1} soil was given during the experiment. These fertilizer rates were chosen to provide adequate nutrients for the growth of the *myc*$^-$ peas, while maintaining good mycorrhizal colonization of *myc*$^+$ roots (Abbott and Robson 1978), and inorganic N was added to reduce N_2 fixation in *myc*$^+$ roots (Jensen 1986). The pots were arranged in a randomized complete block design and placed in a greenhouse with a 12-12 h light-dark

cycle and a mean temperature of 20 °C (day) and 12 °C (night). The moisture was kept at 50% WHC by weighing and adding the water lost regularly twice a week.

Soil samples of 60 g fresh weight were taken from the pots at day 0 and at day 9 (before sowing) for the determination of microbial biomass as well as $\delta^{13}C$ and $\delta^{15}N$ values in soil and microbial biomass. At flowering, root samples from planted pots were taken to 10 cm depth with a soil corer of 3 cm diameter for determination of mycorrhizal colonization. Root samples from all pots of the same treatment were combined and then divided into three parts to obtain a sufficient and equal quantity of material.

Aboveground plant biomass was harvested after a 100-day experimental period on 10 February 2010, separated into straw and seeds and dried. Then, the roots were gently separated from soil and washed thoroughly with water. Fresh root samples of 1.5 and 0.5 g were taken for measuring mycorrhizal colonization and ergosterol, respectively. The other root material was dried. The soil in each pot was thoroughly mixed and a sample of approximately 800 g was taken and sieved through a 2 mm mesh sieve. Pea roots and maize residues > 2 mm were separated with tweezers. These maize residues were the coarse (> 2 mm) particulate organic matter (POM) fraction. A sub-sample of this soil (400 g) was used for determination of the fine (0.4-2 mm) POM fraction. The remaining soil was adjusted to 50% WHC and stored at 4°C until the biological analyses started. All plant parts were oven dried at 60°C for 48 h, weighed and milled for elemental and isotopic analysis.

4.2.3. Microbial biomass C, N, and P

Microbial biomass C and N were estimated using the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). Moist soil of 20 g was split into two portions of 10 g. One portion was fumigated for 24 h with ethanol-free $CHCl_3$. After its removal, the soil was extracted with 40 ml of 0.05 M K_2SO_4 (Potthoff et al. 2003) by 30 min horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The non-fumigated portion was extracted in the same way. Organic C in the K_2SO_4 extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C was E_C/k_{EC}, where E_C = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al. 1990). Total N in the extracts was measured by chemoluminescence detection after

combustion using a Dimatoc 100 automatic analyzer. Microbial biomass N was E_N/k_{EN}, where E_N = (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al. 1985).

Soil microbial biomass P was measured by fumigation extraction (Brookes et al. 1982). Three portions equivalent to 2.5 g oven-dry soil were each extracted with 50 ml of 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 150 rev min^{-1}, centrifuged for 10 minutes at 2,000 xg and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The first portion was fumigated (see above), the second portion was non-fumigated, and the third portion was used for estimating the recovery of 25 µg P g^{-1} soil added as KH_2PO_4 to the extractant. Phosphorus was analyzed by an ammonium molybdate-ascorbic acid method as described by Joergensen et al. (1995). Microbial biomass P was E_P / k_{EP} / recovery, where E_P = (PO_4-P extracted from fumigated soil) - (PO_4-P extracted from non-fumigated soil) and k_{EP} = 0.40 (Brookes et al. 1982).

4.2.4. Ergosterol and amino sugars

Ergosterol was extracted and measured according to Djajakirana et al. (1996). Moist root samples of 0.5 g were extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1}. Ergosterol was measured by reversed-phase HPLC analysis with a mobile phase of 100% methanol and a resolution of detection of 282 nm. The amino sugars muramic acid and glucosamine were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011). A sample of 500 mg root material was weighed into a 20 ml test tube, mixed with 10 ml 6 M HCl, and heated for 3 h at 105°C. From the filtered hydrolysates, a 0.3 ml aliquot was evaporated at 40 °C to dryness. After HCl removal from the filtered hydrolysates and centrifugation at 5000 xg, the sample was transferred to vials and frozen at –18°C until the HPLC measurement. After derivatization with ortho-phthaldialdehyde (OPA), fluorometric emission of amino sugars was measured at a wavelength of 445 nm after excitation at a wavelength of 330 nm. Fungal glucosamine was recalculated into fungal C and muramic acid into bacterial C using the procedure and conversion values proposed by Appuhn and Joergensen (2006) and Engelking et al. (2007).

4.2.5. Mycorrhizal colonization

Fresh root material of 1.5 g was cut into 1 cm lengths and cleared in 10% KOH for 60 min at 65°C. After rinsing with tap water, the samples were acidified with 2 M HCl for 20 min and then stained with 0.1% trypan blue in 90% lactic acid for 20 min at 65°C (Phillips and Hayman 1970; Kleikamp and Joergensen 2006). Subsequently, the roots were distained with lactic acid and stored in a solution containing lactic acid, glycerol, and water (1:1:1 by vol.) until examination. The percentage of root length colonized was determined with the gridline intersection method (Giovannetti and Mosse 1980), using a binocular microscope at × 40 magnification.

4.2.6. Particulate organic matter (POM)

Particulate organic matter was determined according to Magid and Kjaergaard (2001) as described by Muhammad et al. (2006). Moist soil of 400 g was dispersed in 500 ml of 5% NaCl, shaken by hand, and allowed to stand for 45 min. Then the samples were poured gradually onto a sieve of 400 μm mesh size and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve until the water passing through the sieve became clear. The material retained on the sieve was transferred into a bucket. Tap water was added, the bucket was swirled, and organic material was separated repeatedly from the mineral material by flotation-decantation, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining particulate organic matter POM > 400 μm was transferred to a crucible, dried at 60 °C, weighed, and ground for analysis.

4.2.7. Elemental and isotopic analysis

Total C and total N as well as the isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in soil, plant material (straw, root, and seeds), the two POM fractions (0.4-2 and >2 mm), and freeze-dried 0.05 M K_2SO_4 extracts were determined using Delta plus isotope ratio mass spectrometry (Finnigan, Bremen, Germany). The concentrations of P, S, K, Ca, Mg, Na, Fe, Mn, and Al were determined in seeds and straw using HNO_3 pressure digestion (Chander et al. 2008) and inductively coupled plasma atomic emission spectrometry (Spectro Analytical Instruments, Kleve, Germany).

4.2.8. Calculations and statistical analysis

For estimating N_2 fixation in Frisson, the non-nodulated P2 was used as a reference plant. In the *maize*$^+$ treatments, the proportion of plant N derived from fixation (% Ndfa) was calculated using the ^{15}N isotope dilution equation (Ruschel et al. 1979):

$$\%Ndfa = 100 \times \left\{ 1 - \frac{atom\%^{15}Nexcess_{nodulated Frisson}}{atom\%^{15}Nexcess_{non-nodulated P2}} \right\}$$

whereas in the *maize*$^-$ treatments, the natural abundance technique was used (Shearer and Kohl 1986):

$$\%Ndfa = 100 \times \left\{ \frac{\delta^{15}N_{non-nodulated P2} - \delta^{15}N_{nodulated Frisson}}{\delta^{15}N_{non-nodulated P2} - B} \right\}$$

B is the ^{15}N natural abundance for pea, relying on atmospheric N_2 as the sole N source, and was taken as –0.72‰ (Hauggaard-Nielsen et al. 2003).

The enrichment of microbial biomass C with ^{13}C ($\delta^{13}C_{MB}$) was calculated according to Potthoff et al. (2003):

$$\delta^{13}C_{MB} = \frac{(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{nonfum} \times C_{nonfum})}{(C_{fum} - C_{nonfum})}$$

where $\delta^{13}C_{fum}$ and $\delta^{13}C_{nonfum}$ are the $\delta^{13}C$ values of the fumigated and the non-fumigated extract, respectively. C_{fum} and C_{nonfum} are the C content of the fumigated and the non-fumigated extract, respectively. The same equation was used for determination of microbial biomass $\delta^{15}N$ (Dijkstra et al. 2006):

$$\delta^{15}N_{MB} = \frac{(\delta^{15}N_{fum} \times N_{fum} - \delta^{15}N_{nonfum} \times N_{nonfum})}{(N_{fum} - N_{nonfum})}$$

where $\delta^{15}N_{fum}$ and $\delta^{15}N_{nonfum}$ are the $\delta^{15}N$ values of the fumigated and the non-fumigated extract, respectively. N_{fum} and N_{nonfum} are the N content of the fumigated and the non-fumigated extract, respectively. The amount of maize-derived C (C_4-C_{sample}) was calculated for each single replicate of all treatments by the following equation (Balesdent and Mariotti 1996):

$$C_4 - C_{sample} = Ct_{sample} \times \frac{\delta^{13}C_{sample} - \delta^{13}C_{control}}{\delta^{13}C_{maize} - \delta^{13}C_{control}}$$

$$C_3 - C_{sample} = Ct_{sample} - C_4 - C_{sample}$$

where Ct_{sample} is the total C in the analyzed sample, $\delta^{13}C_{sample}$ is the isotopic value ($\delta^{13}C$) in soil organic C, the two POM-C fractions, and microbial biomass C in the treatments with maize residue amendment, $\delta^{13}C_{control}$ is the isotopic value in each single replicate of

the respective fractions in the respective treatments that did not receive maize residues, and $\delta^{13}C_{maize}$ is the isotopic value of the maize residues. Accordingly, the amount of maize-derived N (C_4-N_{sample}) was calculated for each single replicate of all treatments by the following equation (Zareitalabad et al. 2010):

$$C_4 - N_{sample} = Nt_{sample} \times \frac{\delta^{15}N_{sample} - \delta^{15}N_{control}}{\delta^{15}N_{maize} - \delta^{15}N_{control}}$$

$$C_3 - N_{sample} = Nt_{sample} - C_4 - N_{sample}$$

where Nt_{sample} is the total N in the analyzed sample, $\delta^{15}N_{sample}$ is the isotopic value ($\delta^{15}N$) in plant material (straw, seed and root), soil total N, the two POM-N fractions and microbial biomass N in the treatments with residue amendment, $\delta^{15}N_{control}$ is the isotopic value in each single replicate of the respective fractions in the respective treatments that did not receive maize residues, and $\delta^{15}N_{maize}$ is the isotopic value of the maize residues. The microbial residues, which comprise microbial exoenzymes, mucous substances and dead microbial tissue (Khan et al. 2010), were calculated as maize derived soil organic C without POM-C and maize-derived microbial biomass C.

Statistical analyses were carried out using SPSS statistical software (SPSS 15.0). The significance of experimental effects was tested by a two-way ANOVA in tables, and by a one-way ANOVA in figures using the least significant difference (LSD) test ($P<0.05$). The results presented in tables and figures are arithmetic means and are given on an oven-dry basis (about 24 h at 105 °C for soil samples and about 48 h at 60°C for plant parts).

4.3. Results

4.3.1. Microbial root colonization

Myc$^+$ roots were generally well colonized with AM fungi at flowering (Table 4.1). *Myc*$^+$ roots grown in residue-amended soil were significantly less colonized than those grown in non-amended soil. The level of AM colonization strongly decreased, and the difference between the *maize*$^+$ and *maize*$^-$ treatments disappeared at harvest. No AM infection was observed in the *myc*$^-$ roots, except for traces at flowering stage, due to roots from herbs occasionally growing in the pots. The ergosterol content in the root material varied around 111 µg g^{-1} DW without any treatment effect. In contrast, the content of muramic acid was significantly higher in *myc*$^+$ than in the *myc*$^-$ roots,

especially in the *maize*$^{+}$ treatment, whereas glucosamine was generally significantly higher in the *maize*$^{+}$ than in the *maize*$^{-}$ treatment.

4.3.2. Plant responses

Total C yields of straw, grain, and roots of *myc*$^{+}$ plants were significantly higher by 27%, 11% and 92%, respectively, compared with those of *myc*$^{-}$ plants, irrespective of the presence of maize residues (Table 4.2). Straw and grain, but not root yields of *myc*$^{+}$ and *myc*$^{-}$ were on average significantly 14% higher in the *maize*$^{+}$ than in the *maize*$^{-}$ treatment. The $\delta^{13}C$ values in the different plant parts were not influenced by maize application but by pea genotype, being significantly higher in *myc*$^{+}$ than in *myc*$^{-}$ tissue with and without maize (Table 4.3).

Table 4.1: AMF colonization of *myc*$^{-}$ and *myc*$^{+}$ peas at flowering and harvest, grown for 91 days in soil without (*maize*$^{-}$) and with (*maize*$^{+}$) maize residue application; ergosterol, muramic acid and glucosamine concentration in pea roots at harvest.

Treatment	AMF colonization (%)		Ergosterol	Muramic acid	Glucosamine
	Flowering (55 DAS)	Harvest (91 DAS)	($\mu g\ g^{-1}$ DW)		
Myc^{-} *maize*$^{-}$	1	0	106	200	2450
Myc^{-} *maize*$^{+}$	1	0	100	185	2480
Myc^{+} *maize*$^{-}$	47	10	122	213	2140
Myc^{+} *maize*$^{+}$	38	12	116	325	2980
Probability values					
Myc			NS	0.01	NS
Maize	0.05	NS	NS	0.04	0.04
Myc × *maize*			NS	0.01	0.07
CV (± %)	12	32	24	18	16

AMF= arbuscular mycorrhizal fungi; DAS = days after sowing; DW = dry weight; CV = mean coefficient of variation between replicate measurements (n = 3) at flowering and replicate pots at harvest (n = 4); NS = not significant.

Application of labeled maize residues generally resulted in ^{15}N enrichment in the different plant parts (Table 4.3). The $\delta^{15}N$‰ values in straw, seeds and roots of *myc*$^{+}$ plants were significantly lower by 15, 19 and 9‰, respectively, than those of *myc*$^{-}$ plants. In the absence of maize residues, the $\delta^{15}N$ values in the different plant parts did

not differ significantly between the *myc*$^-$ and *myc*$^+$ plants according to the LSD post-hoc test ($P < 0.05$). The yield of C_3-N (N derived from soil, fertilizer, and N_2 fixation) in the plant material was not influenced by maize addition, but it was significantly higher in the straw, seeds and roots of *myc*$^+$ plants by 36, 21 and 220%, respectively, compared with *myc*$^-$ plants, irrespective of the presence of maize residues (Table 4.2). The yields of C_4-N recovered in whole plants in the treatments *myc*$^+$ *maize*$^+$ and *myc*$^-$ *maize*$^+$ were 9.9 and 11.2 mg pot^{-1}, respectively, corresponding to 5.4 and 8.1% of total plant N. The proportion of plant N derived from fixation of the *myc*$^+$ Frisson was 29% or 53 mg pot^{-1} in the *maize*$^+$ treatment but only 5% or 8 mg pot^{-1} in the *maize*$^-$ treatment.

Table 4.2: Contents of total C, C_3-N and maize-derived C_4-N in straw, seeds and roots of *myc*$^-$ and *myc*$^+$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize residues.

Treatment	Total C (g pot^{-1})			Total N (mg pot^{-1})					
				C_3-N			C_4-N		
	Straw	Seeds	Roots	Straw	Seeds	Roots	Straw	Seeds	Roots
Myc$^-$ *maize*$^-$	0.9	1.1	0.3	24.4	95	11.2			
Myc$^-$ *maize*$^+$	1.0	1.2	0.3	21.6	94	10.5	1.8	8.3	1.1
Myc$^+$ *maize*$^-$	1.1	1.3	0.6	29.2	113	25.0			
Myc$^+$ *maize*$^+$	1.3	1.4	0.5	33.2	116	23.0	2.1	5.9	1.9
Probability values									
Myc	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	NS	<0.01	<0.01
Maize	0.04	0.03	NS	NS	NS	NS			
Myc × *maize*	NS	NS	NS	NS	NS	NS			
CV (± %)	13	9	15	16	7	13	11	12	22

CV = mean coefficient of variation between replicate pots (n = 4); NS = not significant.

The element concentrations in straw and seeds were not affected by maize addition, with the exception of K in straw (Table 4.4). Irrespective of the presence of maize residues, straw concentrations of N, Ca, and Mg were significantly higher by roughly 11% in *myc*$^+$ than in *myc*$^-$ plants. Conversely, the straw K concentration was significantly higher in *myc*$^-$ plants. Seed concentrations of N, K, and Fe were also significantly higher by 7, 4 and 16% in *myc*$^+$ plants, whereas P and S concentrations did not differ between the isolines.

Table 4.3: $\delta^{13}C$ and $\delta^{15}N$ values in straw, seeds and roots of *myc*$^-$ and *myc*$^+$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize residues.

Treatment	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Straw	Seed	Roots	Straw	Seeds	Roots
Myc$^-$ *maize*$^-$	-28.5	-26.5	-28.0	13	7	3
Myc$^-$ *maize*$^+$	-29.1	-26.9	-27.9	60	55	58
Myc$^+$ *maize*$^-$	-28.3	-25.5	-27.4	10	8	4
Myc$^+$ *maize*$^+$	-28.5	-25.9	-27.4	45	36	49
Probability values						
Myc	0.07	<0.01	0.05	<0.01	<0.01	0.09
Maize	NS	NS	NS	<0.01	<0.01	<0.01
Myc × *maize*	NS	NS	NS	NS	<0.01	0.07
CV (± %)	2	2	2	24	10	20

CV = mean coefficient of variation between replicate pots (n = 4); NS = not significant.

4.3.3. Microbial biomass

In the unplanted soil, maize residue application caused an initial increase in microbial biomass C of 85 µg g^{-1} soil (Fig. 4.1a). About 70 µg g^{-1} soil of this increase was maize- derived C_4-C, corresponding to 6.5% of added C. This fraction significantly declined to 3.6% at day 9 and increased again to the initial value at day 100. Only 1.6 µg g^{-1} soil or 4% of the added maize C_4-N was incorporated into the microbial biomass at day 0 (Fig. 4.1b). This fraction significantly increased to 7% at day 9 and to 13% at day 100. At each sampling day, maize residue application also caused an additional increase in the soil-derived fraction compared with the *maize*$^-$ treatment (Fig. 4.1; Table 4.5). This increase rose slightly for microbial biomass C_3-C and strongly for microbial biomass C_3-N. The presence of pea plants generally led to a significant increase in microbial biomass C_3-C and C_3-N (Table 4.5). In contrast, pea plants generally had no effects on microbial biomass P, which increased continuously throughout the experiment (Fig. 4.2). This increase was intensified by maize residue application, leading to 42, 33, and 18% higher microbial biomass P contents at day 0, 9 and 100, respectively, in comparison with the *maize*$^-$ treatment.

Table 4.4: Nutrient concentration in straw and seeds of *myc*$^-$ and *myc*$^+$ peas at harvest, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize residues.

Treatment	N	P	S	K	Ca	Mg	Fe
	(mg g^{-1} DW)						
Straw							
Myc$^-$ *maize*$^-$	10.8	0.40	0.71	20.5	21.5	3.94	0.097
Myc$^-$ *maize*$^+$	10.0	0.36	0.75	23.0	21.6	3.99	0.010
Myc$^+$ *maize*$^-$	11.4	0.38	0.71	20.0	23.9	4.40	0.082
Myc$^+$ *maize*$^+$	11.6	0.38	0.73	19.9	24.2	4.34	0.010
Probability values							
Myc	0.07	NS	NS	<0.01	<0.01	<0.01	NS
Maize	NS	NS	NS	0.05	NS	NS	NS
Myc × *maize*	NS	NS	NS	0.03	NS	NS	NS
CV (± %)	8	11	7	5	5	6	21
Seeds							
Myc$^-$ *maize*$^-$	36.2	2.47	1.47	10.0	0.89	1.11	0.084
Myc$^-$ *maize*$^+$	35.4	2.43	1.44	10.0	0.90	1.12	0.078
Myc$^+$ *maize*$^-$	39.0	2.28	1.48	10.5	1.06	1.15	0.086
Myc$^+$ *maize*$^+$	37.8	2.27	1.47	10.4	1.01	1.13	0.102
Probability values							
Myc	0.02	NS	NS	0.01	NS	NS	<0.01
Maize	NS	NS	NS	NS	NS	NS	NS
Myc × *maize*	NS	NS	NS	NS	NS	NS	<0.01
CV (± %)	5	9	5	2	15	3	10

CV= mean coefficient of variation between replicate pots (n = 4); NS = not significant

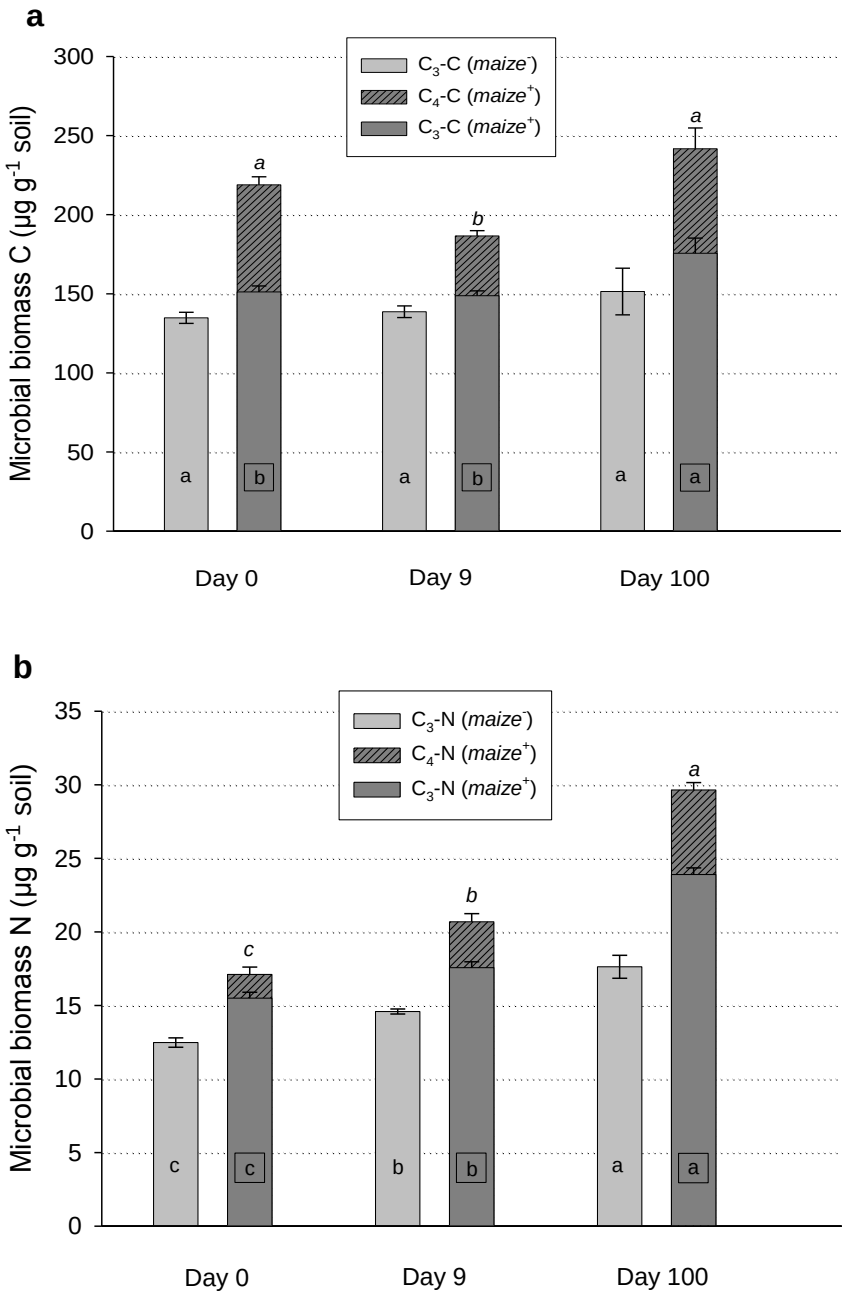

Fig.4.1: Contents of (a) microbial biomass C_3-C and C_4-C as well as (b) microbial biomass C_3-N and C_4-N in unplanted soil (*pea*$^-$), without (*maize*$^-$) and with (*maize*$^+$) maize residues at day 0, immediately after maize application, at day 9 immediately before sowing of peas, and at the end of the 100-day pot experiment; vertical bars show ± one standard error (n = 12 at day 0 and day 9, n = 4 at day 100); different letters above the columns indicate a significant difference for the maize-derived microbial biomass C or N, different letters in squares within the bars indicate a significant difference for the soil-derived microbial biomass C or N in the *maize*$^+$ treatments, and different letters in bars indicate a significant difference for the soil-derived microbial biomass C or N in the *maize*$^-$ treatments according to the LSD test ($P < 0.05$).

Table 4.5: Contents of C_3-C and C_3-N within soil organic matter, microbial biomass and particulate organic matter (POM) in pots without peas (*pea*$^-$), without (*maize*$^-$) and with (*maize*$^+$) maize residues, in pots with *myc*$^-$ and *myc*$^+$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize residues at the end of the 100-day pot experiment.

Treatment	C_3-C				C_3-N			
	Total C (mg g^{-1} soil)	Microbial Biomass C	POM-C (0.4-2mm)	POM-C (>2 mm)	Total N (mg g^{-1} soil)	Microbial biomass N	POM-N (0.4-2 mm)	POM-N (>2 mm)
		(µg g^{-1} soil)				(µg g^{-1} soil)		
Pea$^-$ *maize*$^-$	8.17	152	25		0.95	17.7	1.3	
Pea$^-$ *maize*$^+$	8.15	176	41	19	0.96	24.0	2.1	0.8
Myc$^-$ *maize*$^-$	8.07	190	44		0.89	20.6	2.8	
Myc$^-$ *maize*$^+$	7.83	208	65	28	0.93	25.6	3.4	2.0
Myc$^+$ *maize*$^-$	8.09	171	41		0.89	21.0	2.5	
Myc$^+$ *maize*$^+$	8.01	204	57	37	0.94	23.3	3.4	2.7
Probability values *pea*$^-$ / *pea*$^+$								
Peas	NS	0.01	<0.01	0.01	<0.01	0.01	<0.01	<0.01
Maize	NS	0.01	<0.01	-	0.01	<0.01	<0.01	-
Peas × *maize*	NS	NS	NS	-	0.06	NS	NS	-
Probability values *myc*$^-$ / *myc*$^+$								
Myc	NS	NS	0.07	0.09	NS	NS	NS	NS
Maize	NS	0.02	<0.01	-	0.01	0.09	0.01	-
Myc × *maize*	NS	NS	NS	-	NS	NS	NS	-
CV (± %)	4	11	10	18	2	12	12	16

CV= mean coefficient of variation between replicate pots (n = 4); NS = not significant.

At the end of the experiment, the contents of maize-derived microbial biomass C_4-C and C_4-N were highest in the unplanted soil and were reduced by 9% and 12%, respectively, in the presence of *myc*$^-$ plants and significantly by 17% and 22%, respectively, in the presence of *myc*$^+$ plants (Table 4.6).

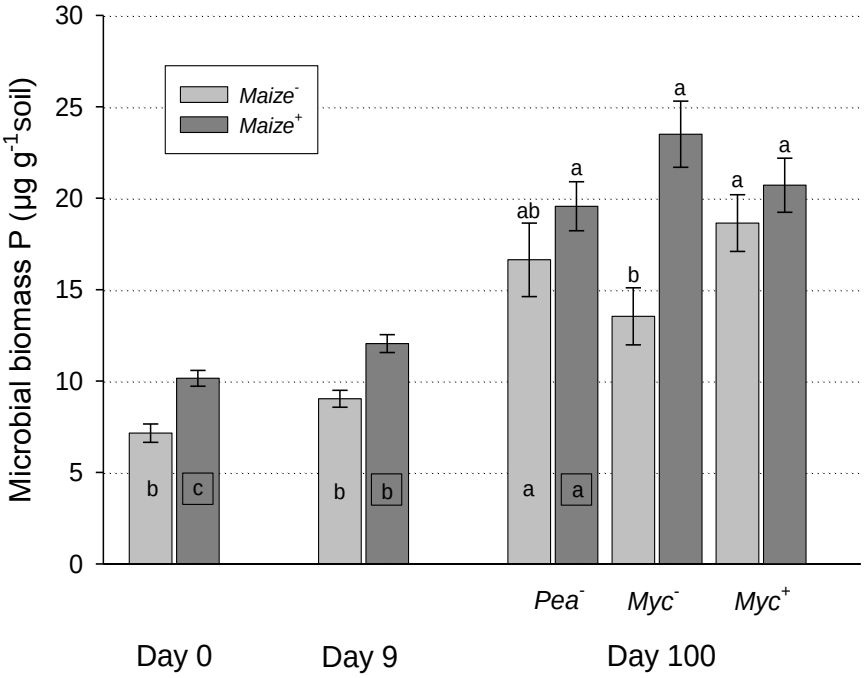

Fig.4.2: Contents of microbial biomass P in the soil without (*maize*$^-$) and with (*maize*$^+$) maize-residues at day 0, immediately after maize addition, at day 9 immediately before sowing of peas; contents of microbial biomass P in unplanted soil (*pea*$^-$) as control and *myc*$^-$ and *myc*$^+$ peas at the end of the 100-day pot experiment; vertical bars show ± one standard error (n = 12 at day 0 and day 9, n = 4 at day 100); different letters above the bars indicate a significant difference at the end of the experiment, different letters in squares within bars indicate a significant difference in the *maize*$^+$ treatments, and different letters in bars indicate a significant difference in the *maize*$^-$ treatments, according to the LSD test ($P < 0.05$).

4.3.4. Particulate organic matter

Maize residue application significantly increased the content of soil-derived C_3-C in the fine POM-C fraction by 64, 48 and 39% in the *pea*$^-$, *myc*$^-$, and *myc*$^+$ treatments, respectively, compared with the *maize*$^-$ treatments (Table 4.5). The same was true for soil-derived C_3-N in the fine POM-N fraction. The corresponding increases were 62, 21 and 36%, respectively. The presence of pea plants led to a significant increase in soil-derived C_3-C and C_3-N in the coarse POM fraction, especially in the *myc*$^+$ treatment.

Maize- derived C_4-C and C_4-N of the fine POM fraction increased significantly by 60 and 93%, respectively, in the *pea*$^+$ compared with the *pea*$^-$ treatments (Table 4.6).

This fraction was not affected by mycorrhizal presence. Maize -derived C_4-C and C_4-N of the coarse POM fraction significantly differed between the three *maize*$^+$ treatments. They showed a significant 2.4- and 3-fold increase, respectively, in the presence of *myc*$^-$ roots and a significant 3.3- and 4.3-fold increase in the presence of *myc*$^+$ roots. The recovery of maize residue C and N in the two POM fractions was roughly 6% in the *pea*$^-$ soil and increased to 14% in the presence of *myc*$^-$ and 18% in the presence of *myc*$^+$ roots (Fig. 4.3).

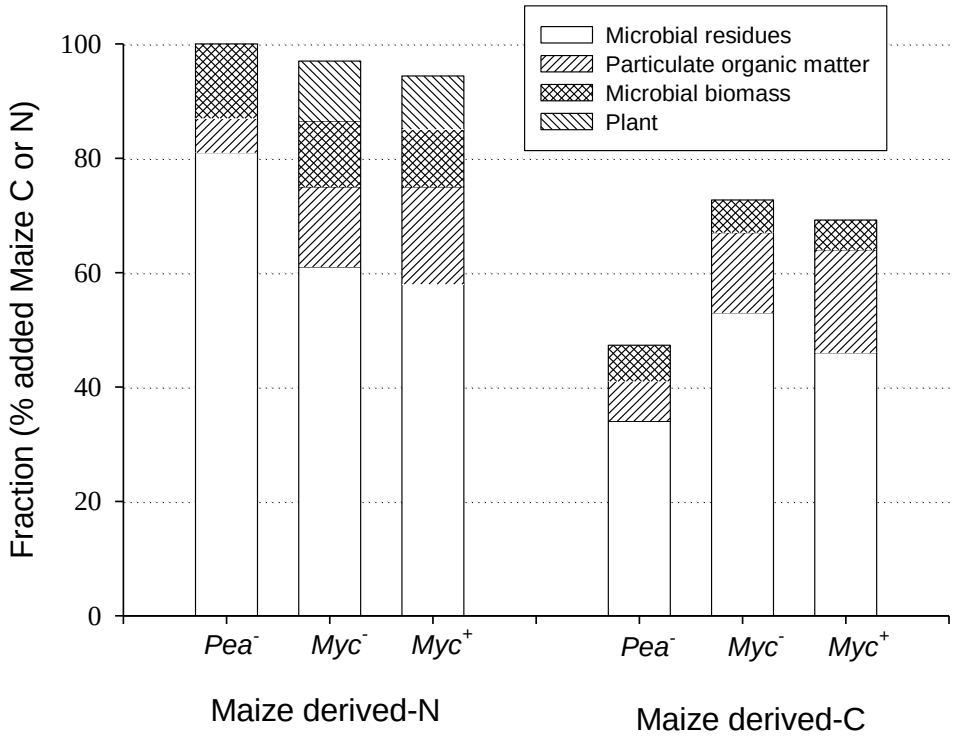

Fig.4.3: Distribution of maize residue-N within microbial residues N, microbial biomass N, particulate organic matter N, and plant-N as well as distribution of maize residue-C within microbial residues C, microbial biomass C, particulate organic matter C in unplanted soil (*pea*$^-$) with maize residues (*maize*+), the *myc*$^-$ *maize*$^+$ treatment and *myc*$^+$ *maize*$^+$ treatment at the end of the 100-day pot experiment.

Table 4.6: Contents of maize-derived C_4-C and C_4-N within soil organic matter, microbial biomass and particulate organic matter (POM) in pots without peas (*pea*$^-$), without (*maize*$^-$) and with (*maize*$^+$) maize residues, in pots with *myc*$^-$ and *myc*$^+$ peas, grown in soil without (*maize*$^-$) and with (*maize*$^+$) maize residues at the end of the 100-day pot experiment, *myc*$^-$ and *myc*$^+$ peas were both inoculated with AMF.

Treatment	C_4-C				C_4-N			
	Total C (mg g^{-1} soil)	Microbial biomass C (µg g^{-1} soil)	POM-C (0.4-2mm)	(>2 mm)	Total N (mg g^{-1} soil)	Microbial biomass N (µg g^{-1} soil)	POM-N (0.4-2 mm)	(>2 mm)
Pea$^-$ *maize*$^+$	0.45 b	66 a	31 b	42 c	0.043 a	5.8 a	1.5 b	1.1 c
Myc$^-$ *maize*$^+$	0.67 a	60 ab	50 a	102 b	0.035 b	5.1 ab	3.0 a	3.3 b
Myc$^+$ *maize*$^+$	0.59 ab	55 b	49 a	140 a	0.033 b	4.5 b	2.8 a	4.7 a
Probability values								
Pea$^-$ / *pea*$^+$	0.01	0.07	0.01	<0.01	<0.01	0.02	<0.01	<0.01
Myc$^-$ / *myc*$^+$	NS	NS	NS	0.02	NS	0.06	NS	0.01
CV (± %)	20	15	17	18	8	9	17	14

CV = mean coefficient of variation between replicate pots (n = 4); different letters within a column indicate a significant difference ($P < 0.05$; LSD post-hoc test, n = 4); NS = not significant.

4.4. Discussion

4.4.1. Plant responses

The amount of inorganic fertilizer added was sufficient for the growth of *myc*$^-$ pea mutant P2, but its yield components did not reach the levels of *myc*$^+$ Frisson. In general, the N_2 fixation of Frisson was low, due to the inorganic N fertilizer addition. However, the presence of maize residues alleviates this negative effect of N on N_2 fixation, probably due to microbial immobilization of available inorganic N (Heckman and Kluchinski 1995). This immobilization may also explain the lower N concentration of P2 plants in the *maize*$^+$ compared to *maize*$^-$ treatment. The root weight of *myc*$^+$ plants was almost twice that of *myc*$^-$ plants. AMF are known to affect shoot-root assimilate allocation patterns, leading to an increased root growth, as observed by Jackson et al. (2002). Although the N concentration was slightly lower in straw and seeds of *myc*$^-$ peas, the concentrations of P and the other nutrients were similar in the different plant parts of the two isolines and did not indicate any nutrient-specific difficulties in uptake for *myc*$^-$ peas. Although the mutation should only affect the genes for mycorrhizal and rhizobial infection (Duc et al. 1989), further genetic defects cannot be fully excluded (Kleikamp and Joergensen 2006). Interactions with plant hormones may be involved in the mutation, as suggested by experiments with mutant P2 treated with an auxin-transport inhibitor (Müller 1999).

The $\delta^{13}C$ values in the seeds of both pea isolines are similar to those of *Phaseolus vulgaris* seeds (Bathellier et al. 2008). In the different parts of both pea isolines, the $\delta^{13}C$ values decreased in the order seeds > roots > straw, supporting the view of Bathellier et al. (2008) that post-photosynthetic fractionations occur in plants, leading to ^{13}C enrichment in heterotrophic organs such as roots in comparison with autotrophic organs such as the shoot (Voisin et al. 2003a). Nodules were usually the sink with the highest demand for C (Voisin et al. 2003b). However, the nodules were present on Frisson roots at harvest, although they were scarce and small. The maize residue application did not affect $\delta^{13}C$ values, suggesting that the mineralization of maize leaves did not contribute significant amounts to the photosynthetic CO_2 uptake of the pea plants. An interesting, unknown feature is the observation that the plant parts of mutant P2 almost always contained significantly less $\delta^{13}C$ and consequently a stronger $^{13}C/^{12}C$ fractionation. One explanation is the higher depletion of $\delta^{13}C$ by dark respiration (Bathellier et al. 2008). Another likely explanation is

a higher water use efficiency of the pea plants in the presence of AMF, leading to higher $\delta^{13}C$ values in plant tissues (Handley et al. 1993; Newton et al. 1996).

Also the $\delta^{15}N$ values varied significantly between the different plant parts. They decreased in the order straw > seeds > roots in the *maize*$^-$ treatment. This fractionation indicates an enrichment of ^{15}N in autotrophic shoot in comparison to the heterotrophic roots. No data are available for direct comparison. However, the distribution of a ^{15}N label in the different plant organs of pea plants was in the same order as that observed by Wichern et al. (2007). In leaves, the transfer of glutamine N to glutamic acid is accelerated in the light, and amino N in some amino acids is deaminated to ammonia in the dark, followed by its incorporation into glutamine (Yoneyama et al. 2003). This more intensive metabolism leads to enrichment during maturation, after the export of N components relatively depleted in ^{15}N. In the *maize*$^-$ treatment, no differences occurred between the two isolines. In contrast, the $\delta^{15}N$ values in the *maize*$^+$ treatment tended to be significantly lower in the *myc*$^+$ plants, indicating a lower uptake of maize-derived N caused by the lower decomposition rate of maize residues in this treatment. AMF provides plants with an extensive root system, exploring more soil volume by external hyphae (Smith and Read 2008). Mycorrhizal hyphae absorb N from the soil, which is not normally available to non-mycorrhizal roots (e.g. from autochthonous C_3-N sources, which have lower^{15}N enrichment). They can absorb inorganic N, particularly immobile NH_4^+, and transport it to their host plant (Maeder et al. 2000). Another explanation may be the contribution of the unlabeled N from fixation of atmospheric N_2 (Boddey et al. 1990).

4.4.2. Microbial root colonization

Mutant P2 has been intensively studied for its defense reactions against AMF (Ruiz-Lozano et al. 1999), so that it was expected that roots of the *myc*$^-$ mutant P2 were not colonized by AMF throughout the experiment. In contrast, *myc*$^+$ roots were highly colonized at flowering by AMF (Baird et al. 2010), despite the application of N and P fertilizer. A decline of mycorrhizal root colonization during maturation has been observed by others in the field, but it was not as drastic as in the present pot experiment (Vestberg et al. 2010). At the end of the pot experiment (91 DAS), the concentration of ergosterol in roots of mature pea plants did not differ between the two isolines, suggesting similar colonization by saprotrophic fungi. As AMF do not contain ergosterol (Olsson et al. 2003), ergosterol is an important indicator for saprotrophic fungi in arable soils (Joergensen and Wichern 2008). The mean ergosterol concentration of the present experiment was

somewhat above the 72 µg ergosterol g^{-1} DW obtained in *Trifolium alexandrium* roots at 80 DAS, exhibiting a 30% AMF colonization rate (Frey et al. 1992), and was identical to the 111 µg ergosterol g^{-1} DW in the annual legume *Kummerowia striata* at 80 DAS (Fujiyoshi et al. 2000). However, in contrast to our results, Frey et al. (1992) and Fujiyoshi et al. (2000) found significantly higher amounts of ergosterol in mycorrhizal than in non-mycorrhizal roots.

Also the concentration of the cell-wall amino sugars muramic acid and glucosamine indicate a strong microbial colonization of the roots (Appuhn and Joergensen 2006). The mean ratio of fungal glucosamine to ergosterol was 21 and thus within the range of 12 to 41 obtained in different non-AMF Basidiomycota (Matcham et al. 1985; Wallander et al. 1997; Plassard et al. 2000). This suggests that the roots of the present mature pea plants were mainly colonized by saprotrophic fungi. Biotrophic AMF Glomeromycota do not contain ergosterol (Olsson et al. 2003) but glucosamine (Joergensen and Wichern, 2008). The mean ratios of fungal C to bacterial C were 2.3 for myc^- and 1.6 for myc^+ peas, indicating generally fungal dominance for root colonizing microorganisms in accordance with Appuhn and Joergensen (2006). The significant lower fungal C to bacterial C ratio for myc^+ peas was mainly due to the $maize^+$ treatment, suggesting that decomposing maize residues specifically promoted root colonizing bacteria for unknown reasons. AMF can affect the microbial community in the rhizosphere (Wamberg et al. 2003) but also the bacterial community structure on the root, due to an enhanced pH in the presence of mycorrhizal hyphae (Marschner and Baumann 2003) and due to an altered release of carbohydrates to the rhizosphere (Wamberg et al. 2003). Several studies have observed that the presence of AMF can increase root colonization by bacteria (Marschner and Baumann 2003; Miyauchi et al. 2008). However, the strong shift in the mean ratio between myc^- and myc^+ was equivalent to a rather moderate decrease in fungal tissue as a percentage of the total microbial C (fungal C + bacterial C) from 70 to 62%.

4.4.3. Microbial use of maize residues

The application of maize residues had only small effects on plant growth, but strong effects on the microbial biomass. In contrast, plant growth has strong effects on the decomposition of maize residues and the incorporation of maize-derived C and N into the microbial biomass and microbial residues. The decomposition rate of added maize residues was significantly reduced by the presence of peas, especially by myc^+ peas. This reduction was indicated by the higher contents of POM-C and POM-N as well as the lower

incorporation of maize-derived C and N into the microbial biomass. The mean microbial biomass C_4-C/N ratio was 12 in all treatments at the end of the pot experiment, exceeding the mean total microbial biomass C/N ratio of 9 at this time. In contrast, the mean C_4-C/N ratio of microbial residues was 10 in the absence of plants and 19 in their presence, i.e. close to the 24 of the original maize residues. This was caused by an increased formation of microbial residue C and a reduced formation of microbial residue N in the presence of plants. The demand for internal N recycling processes within the microbial cells reduced the decomposition rate of maize residues caused by an insufficient N supply to soil microorganisms. A C/N ratio of 24 of the maize residues is probably too narrow to cause strong N immobilization (Powlson et al. 2001), but it is too wide for maximum microbial growth and decomposition activities. As the pots in the present experiment were regularly watered, it is unlikely that a low soil moisture was an important reason for the reduced decomposition rate of maize residues in the presence of plants, contrasting the view stated by others in field (Christensen 1985) and pot experiments (Muhammad et al. 2007). Two alternative explanations may be considered for this observation: (1) preferential utilization by microorganisms of fresh materials (highly labile rhizodeposition) released from roots than the labeled organic material (Nicolardot et al. 1995) and (2) competition between pea plants and microorganisms for soil resources such as nutrients, especially N (Jingguo and Bakken 1997).

The recovery of maize residues as POM was similar to that of Zareitalabad et al. (2010), who used green maize leaves with a similarly low C/N ratio, and markedly lower than that of Rottmann et al. (2010), who used maize leaf straw with a C/N ratio of 79. If we assume that the balance gap between POM, microbial biomass C and microbial residue C can be fully assigned to CO_2, the yield coefficient Y can be calculated as follows (van Veen et al. 1984; Joergensen et al. 1990):

Y = substrate C in microbial products / utilised substrate C = A / B
A = % microbial biomass C_4-C + microbial residue C_4-C
B = 100 – (% POM-C_4-C)

This calculation results in a yield coefficient Y = 0.43 (A / B = 40% / 93%) of unplanted treatment, Y = 0.68 (A / B = 59% / 86%), and Y = 0.62 (A / B = 51% / 82%) in the presence of *myc*$^-$ and *myc*$^+$ plants, respectively, suggesting a significantly higher substrate use efficiency. These yield coefficients are close to the range of 0.37 to 0.59 obtained by Muhammad et al. (2006) and Rottmann et al. (2010), respectively, for maize leaf straw,

differing in N content and microbial colonization. The present yield coefficients are also close to that of 0.55 for the metabolized remains of the original soil microbial biomass after 10-day incubation (Jenkinson and Powlson 1976). Microbial yield coefficients apparently depend more on the environmental conditions for microbial turnover in soil than on substrate quality. The higher root density of *myc*$^+$ peas might also lower the recovery of the C and N fractions, probably mainly due to an insufficient recovery of POM and roots in these pots. This increases the apparent percentage of utilized substrate by soil microorganisms. However, the error is relatively small and does not affect the overall differences between the *myc*$^-$ and *myc*$^+$ or the *maize*$^-$ and *maize*$^+$ treatments.

A specific feature of the present results is the increase in soil-derived microbial biomass C_3-C and C_3-N after the application of maize residues. This is certainly possible at day 9 and day 100 and might be interpreted as a priming effect caused by the application of an easily available substrate. However, this increase is certainly not possible immediately after application of the maize residues. This suggests that the $\delta^{13}C$ and $\delta^{15}N$ values of the $CHCl_3$ labile fraction derived from litter colonizing microorganisms were lower than in the other fractions of the maize residues. The presence of saprotrophic fungi on maize residues may have caused this reduction in $\delta^{13}C$ and $\delta^{15}N$ values. A fractionation of $\delta^{13}C$ and $\delta^{15}N$ values has been repeatedly observed in saprotrophic fungal biomass (Hobbie et al. 2004). This fractionation was also caused by ecto-mycorrhizal and even by AM fungi, additionally affecting the $\delta^{15}N$ values of their host plants (Hobbie et al. 2008; Hobbie and Hobbie 2008).

Another specific feature is that soil-organic matter derived autochthonous microbial biomass N and microbial biomass P significantly increased over the experimental period in all treatments, i.e. also in the unplanted and non-amended control pots. This increase has sometimes been observed in incubation experiments (Muhammad et al. 2006), but is still not fully understood. One reason might be that soil organic matter is made available, e.g. to saprotrophic fungi (Rost et al. 2001) at a constant relatively high temperature and high moisture levels (Joergensen et al. 1990). In contrast to other pot experiments, plant growth has small but significant increasing effects on the microbial biomass. These differences might be due to the growth stage of the plant. In mature plants, the roots are strongly colonized by fungi, which might lead to a strong C transfer by saprotrophic fungal hyphae, as observed by Butenschoen et al. (2007) and Rottmann et al. (2010) from the detritusphere of leaf litter to the bulk soil.

4.5. Conclusions

The comparison of the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson makes it possible to investigate the additional effects of AMF and growing pea plants (*myc*$^+$ and *myc*$^-$) on the decomposition and microbial use efficiency of ^{15}N-labeled maize residues. Virtually nothing is known about the interactions of saprotrophic soil microorganisms, biotrophic AMF, and plant roots, despite the omnipresence of mycorrhizal symbiosis. The decomposition rate of added maize residues was significantly reduced by the presence of *myc*$^-$ peas, but especially by *myc*$^+$ peas, leading to a decreased turnover of the microbial biomass and an improved microbial substrate use efficiency. The formation of microbial residue C was increased and that of microbial residue N was reduced in the presence of plants. This means that the insufficient N supply to soil microorganisms and not a reduced water availability reduced the decomposition of maize residues in the presence of peas, especially *myc*$^+$ peas. AMF are apparently not involved in the decomposition of newly added organic residues, but intensify the competition between plants and soil microorganisms for available N. This finding needs support and further evidence by additional experiments.

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5. Organic fertilizer effects on pea yield, nutrient uptake, microbial root colonization and soil microbial biomass indices in organic farming systems.

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ABSTRACT

In the present field experiment, horse manure and compost derived from shrub and garden cuttings were supplied at nearly equivalent N amounts but different C amounts to field peas (*Pisum sativum* L.), either as a sole crop or intercropped with oat (*Avena sativa* L.). The objectives were: (1) to evaluate the beneficial effects of C-rich manure and compost on pea productivity in different cropping systems (2) to investigate whether these effects were reflected by microbial root colonization, microbial biomass and CO_2 production and (3) to study the residual effects of the organic fertilizers on the yield of succeeding crop. Short term application of horse manure and compost greatly stimulated soil microbial biomass C, N, P, fungal ergosterol and CO_2 evolution, but failed to stimulate productivity of the current crops. However, significant positive residual effects of organic fertilizer, especially horse manure were observed on the grain yield of the succeeding winter wheat. Mycorrhizal colonization and ergosterol concentration were significantly higher in pea than in oat roots. Intercropping is an important tool for controlling weeds on pea plots under organic farming conditions, but did not affect microbial root colonization, soil microbial biomass indices or CO_2 evolution from the soil surface. According to the extrapolation of the CO_2 evolution rates into amounts per hectare, approximately 40% of the manure C and 24% of the compost C were mineralised to CO_2 during the 124-day

experimental period. There were close relationships between grain N and P concentrations in both crops and microbial biomass C, N and P, suggesting that soil microbial biomass can be used as an indicator of nutrient availability to plants.

Keywords: Horse manure, Compost, Microbial biomass, CO_2 evolution, Peas, Intercropping

5.1. Introduction

In organic farming systems, N_2 fixation of legumes, such as peas (*Pisum sativum* L.) is the main source of N input (Berry et al., 2002). In these systems, peas are usually grown in rotation or intercropped with cereal crops, particularly with summer barley (*Hordeum vulgare* L.; Jensen, 1996), summer wheat (*Triticum aestivum* L.; Ghaley et al., 2005), and oats (*Avena sativa* L.; Neumann et al., 2007). Intercropping of peas with cereals is known to increase yields of an associated (Giller et al., 1991; Jensen, 1996) or a following cereal crop (Chalk et al., 1993). Intercropping is also known to decrease weed pressure (Hauggaard-Nielsen et al., 2001, 2008). Organic fertilizers provide the majority of essential plant nutrients, improving actual crop productivity but also leaving beneficial residual effects on succeeding crops (Ghosh et al., 2004). However, application of C-rich organic fertilizers such as horse manure or yard-waste compost, containing large amounts of bedding straw and woody debris with a wide C/N ratio, may cause temporary N-immobilization (Mahimairaja et al., 1994; Thomsen and Kjellerup, 1997). This will restrict crop productivity, particularly of non-legumes dependent on mineralization of soil organic N (Ramesh et al., 2009; Doltra et al., 2011).

Incorporation of organic fertilizers into soil causes a large and rapid increase in the soil microbial biomass (Ghoshal and Singh, 1995; Heinze et al., 2010), which forms only a small fraction of soil organic matter. However the soil microbial biomass plays an important role in nutrient cycling and plant nutrition, due to its fast turnover (Jenkinson and Ladd, 1981). For this reason, some studies have found a close relationship between the soil microbial biomass and crop yields under greenhouse conditions (Chen et al., 2000) as well as under field conditions (Insam et al., 1991; Goyal et al., 1992; Khan and Joergensen, 2006; Mandal et al., 2007). However, this relationship has not always been observed (Nilsson et al., 2005). Another important index for soil biological activity in the field is

CO_2 evolution (Müller et al., 2011), i.e. the sum of microbial and root respiration (Jensen et al., 1996). Application of organic fertilizers to soil increases CO_2 emissions caused by microbial decomposition processes (Jensen et al., 1997; Terhoeven-Urselmans et al., 2009).

In the present field experiment, horse manure and yard-waste compost, derived from shrub and tree clippings were supplied at nearly equivalent N amounts but different C amounts to field peas (*Pisum sativum* L.), either as a sole crop or intercropped with oats (*Avena sativa* L.). The underlying hypotheses were: (1) C-rich organic fertilizers have beneficial effects on pea productivity in different cropping systems. (2) These beneficial effects are reflected by CO_2 production and microbial biomass indices. The specific objectives were to compare the effects of horse manure and yard-waste compost (a) on microbial indices in soil and roots, and (b) on growth and yield of peas, grown as the sole crop or intercropped with oats. (c) to study the residual effects of the organic fertilizers on wheat as a succeeding crop in organic farming.

5.2. Material and methods

5.2.1. Site and soil

The field experiment was carried out from April to August 2009 for the target crops and from October 2009 to August 2010 for the succeeding wheat crop at Frankenhausen, the experimental farm of the University of Kassel in northern Hessia (51°24' N, 9° 25' E, and 248 m above sea level). Total precipitation for the year 2009 was 528 mm, 170 mm below the long-term average, but evenly distributed throughout the growing season (Fig.5. 1). The mean temperature was 9.2 °C, 0.6 °C above the long-term average and varied between 12 and 18 °C from sowing to harvest. The soil was classified as Haplic Luvisol (Quintern et al., 2006) and contained 17% clay, 81% silt, 2% sand, 1.2% total C and 0.15% total N. The soil pH (H_2O) was 7.2. The preceding crops on the experimental field were a mixture of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) in 2007 and potatoes (*Solanum tuberosum* L.) in 2008.

5.2.2. Experimental design

Semi-leafless field peas (*Pisum sativum* L. var. Santana, KWS, Einbeck, Germany) were grown as the sole crop or intercropped with oats (*Avena sativa* L. var. Dominik). The organic fertilizers used in the experiment were horse manure (mixed with stall bedding) and shredded yard-waste compost, derived from shrub and tree clippings. The following six treatments were performed (1) sole peas, (2) sole peas + manure, (3) sole peas + compost, (4) peas intercropped with oats, (5) peas intercropped with oats + manure and (6) peas intercropped with oats + compost. The experimental plots (6 × 4.5 m) were arranged in a randomised block design with four replicates, separated from each other by a walking path of 0.5 m width. Each plot was divided into three subplots (6 m × 1.5 m): one for soil respiration measurements and soil sampling, one for destructive sampling of plants during the growing period, and one for final yield measurements, where the plants were left undisturbed until harvest. Subplots consisted of seven rows with a row distance of 18.75 cm.

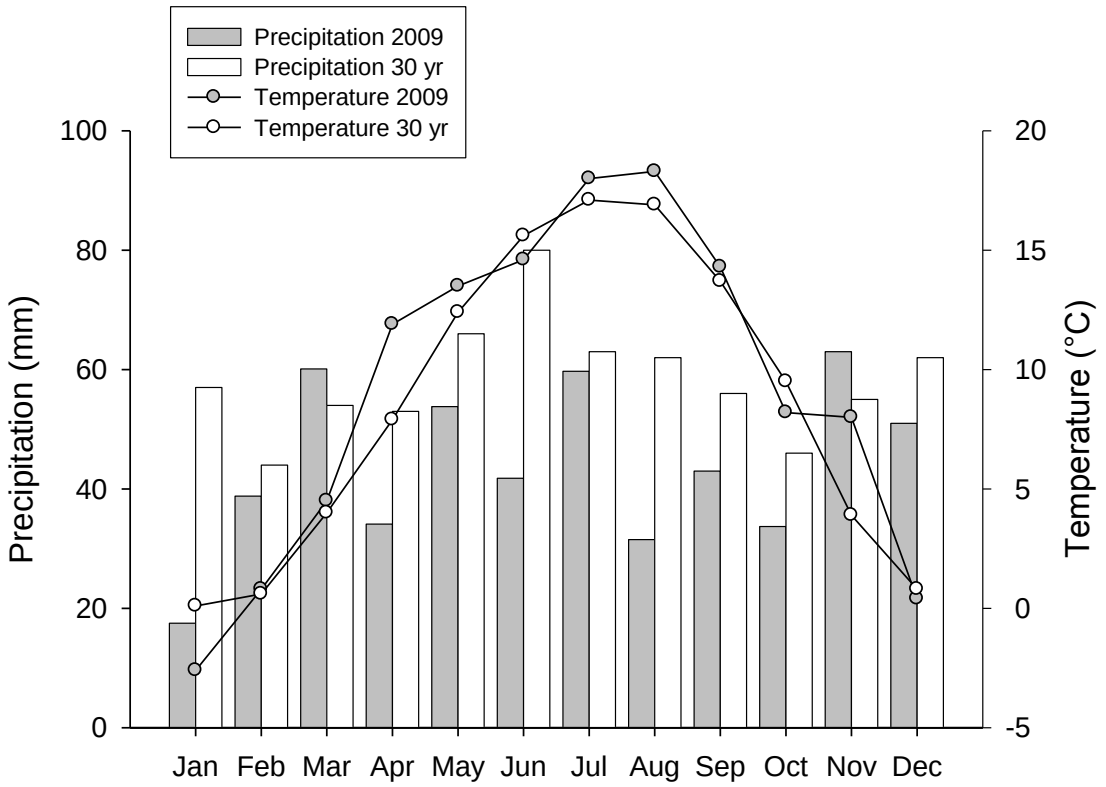

Fig.5.1**:** Mean monthly precipitation (bars) and temperature (line) for the year 2009 as compared to the long-term (1960-1990) at the study site.

The horse manure was applied at the rate of 10 t C and 230 kg N ha^{-1}, while the compost was added at a rate of 5 t C and 290 kg N ha^{-1}. Some properties of the organic fertilisers used are shown in Table 5.1. The site was ploughed to a depth of 25 cm on 15 October 2008 followed by a shallow seedbed preparation on 7 April 2009 using a rotary harrow. Fertilizers were applied by hand on 8 April 2009, and then incorporated to a depth of 18 cm by rotary cultivator. The crops were sown on 9 April 2009. Peas were sown at 80 seeds m^{-2} in both the sole crop and intercropped plots. Oats were sown at 60 kernels m^{-2}. Intercropped peas and oat were sown in the same row. For determining the residual effect of organic fertilizers applied on the yield of the succeeding winter wheat, the experimental plots were ploughed again on 19 October 2009, The post-harvest residues from the first season were incorporated into the soil, followed by a shallow seedbed preparation on 21 October using a rotary harrow. Winter wheat (*Triticum aestivum* L., cv. Achat) was sown on 22 October at a density of 300 seeds m^{-2} and a row distance of 18.75 cm. Plant populations were determined by counting the number of seedlings in three randomly selected 1 m lengths of rows in each plot. The crops were cultivated according to organic agricultural practice with no use of herbicides and with mechanical weeding three times during emergence and leaf development stage. Scarecrows were used in order to reduce damage from birds.

Table 5.1: Some properties of organic fertilizers used in this study

Organic fertilizer	Total C	Total N	Available P	Available K	DW	Applied rate (t ha^{-1})	
	(mg g^{-1})				(%)	DW	FW
Horse manure	453	10.3	0.92	27.1	22	22.1	100
Compost	278	15.8	1.33	11.1	56	18.1	32.2

DW = dry weight, FW = fresh weight

5.2.3. Plant sampling

Crop development was recorded at approximately weekly intervals according to the BBCH code (Meier, 1997). Plant height was measured from the base of the plant to the base of the flag leaf on the main tiller of oat plants and to the tip of the central axis of pea plants. Plant stand heights were measured from the soil surface to the highest point in the canopy at three places in each plot after flowering of peas 75 days after sowing (DAS) and at maturity of peas (127 DAS). Stand height index was calculated as stand height at maturity divided by stand height at flowering (Sauermann, 2007). Above ground dry

matter production was determined at the end of flowering of peas (BBCH 69; 71 DAS) and at the senescence of peas (BBCH 97; 120 DAS). At each harvest, crops were harvested manually by cutting the above-ground plant parts at ground level from 1 m^2 area randomly selected from each plot. The harvested plant biomass was divided into three fractions, i.e. pea, oat and weeds. At the last harvest, grain production of peas and oats was determined after threshing. The succeeding winter wheat was harvested on 20 August 2010. The samples were dried at 60°C for 72 h and weighed for determination of dry weight production.

Three additional randomly selected plants from each plot were cut at the soil surface for determining the yield components per plant, mycorrhizal colonization and ergosterol were taken at the end of flowering of peas (BBCH 69; 71 DAS). Roots were sampled with a soil corer (7 cm diameter × 15 cm depth), washed free of soil with tap water, separated according to the crop species, and bulked within the plots for counting nodules and measuring mycorrhizal colonization and ergosterol. Dry matter of all samples was determined after drying at 60°C for 72 h. Dried plant material was ground and analysed for total C and total N using a Vario Max CN analyser (Elementar, Hanau, Germany). The concentrations of P, S, K, Ca, Mg, Na, Fe, Mn and Al were determined in grain and straw using HNO_3 pressure digestion (Chander et al., 2008) and measured by ICP atomic emission spectrometry (Spectro Analytical Instrument, Kleve, Germany).

5.2.4. Soil sampling

The soil was sampled three times during the growing season, at the early leaf development of peas (BBCH 11; 13 DAS), at the end of flowering of peas (BBCH 69; 71 DAS) and at the senescence of peas (BBCH 97; 120 DAS). From each plot, three samples were collected with a soil corer (7 cm diameter) to a depth of 20 cm at three random points between the rows. Cores from each plot were bulked, mixed thoroughly, and a representative sample of 1 kg was taken. This soil was passed through a 2-mm sieve, and stored in polyethylene bags at 4°C for no longer than 2 weeks until soil biological analysis was carried out.

5.2.5. Photosynthetically active radiation (PAR)

Canopy interception of photosynthetically active radiation (PAR) was measured twice during the growing season, once at early pod development of peas (BBCH 73, 76 DAS)

and once at ripening of peas (BBCH 85, 111 DAS), using a 1 m line quantum sensor (LI-COR, LI-191 SA). Measurements were made by taking two readings above the canopy and four to five readings below the canopy at the soil surface at randomly selected locations within each plot. The percentage PAR intercepted by the canopy (PAR_{int}) was calculated with the following equation (McIntyre et al., 1997):

$$PAR_{\text{int}}(\%) = \left[1 - \frac{PAR_{belowcanopy}}{PAR_{abovecanopy}}\right] \times 100$$

5.2.6. Mycorrhizal colonization and ergosterol

Fresh root material of peas and oats (1.5 g) was cut into 1 cm lengths and cleared in 10% KOH for 60 min at 65°C for peas and at 90°C for oats. After rinsing with tap water, the samples were acidified with 2 M HCl for 20 min, and then stained with 0.1% trypan blue in 90% lactic acid for 20 min at 65°C for peas and at 90°C for oats (Phillips and Hayman, 1970; Kleikamp and Joergensen, 2006). Subsequently the roots were distained with lactic acid and stored in a solution containing lactic acid, glycerol and water (1:1:1 by vol.) until examination. The percentage of root length colonized was determined with the gridline intersection method (Giovannetti and Mosse, 1980) using a binocular microscope at × 40 magnification. Ergosterol was extracted and measured according to Djajakirana et al. (1996). Moist samples of 0.5 g root material and 2 g soil were extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev min^{-1}. Ergosterol was measured by reversed-phase HPLC analysis with a mobile phase of 100% methanol and a resolution of detection of 282 nm.

5.2.7. Soil microbial biomass

Microbial biomass C and microbial biomass N were estimated by the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Fumigated and non-fumigated portions of 10 g moist soil were extracted for 30 min by oscillating shaking at 200 rev min^{-1} with 40 ml 0.5 M K_2SO_4 and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C and total N in the extracts were measured after combustion at 850ºC using a Dimatoc 100 + Dima-N automatic analyser (Dimatec, Essen, Germany). Microbial biomass C was calculated as E_C / k_{EC}, where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N / k_{EN}, where E_N = (total N extracted from

fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al., 1985). The *k* values represent the extractable part of the microbial biomass after fumigation.

Soil microbial biomass P was also measured by the fumigation-extraction method (Brookes et al., 1982) as described by Joergensen et al. (1995). Microbial biomass P was calculated as E_P / k_{EP} / recovery, where E_P = (PO_4^{3-}-P extracted from fumigated soil) - (PO_4^{3-}-P extracted from non-fumigated soil) and k_{EP} = 0.40 (Brookes et al., 1982). Recovery of added P (25 µg g^{-1} soil) to account for P adsorption during extraction was calculated as follows: 1 – ((PO_4^{3-}-P extracted from non-fumigated and spiked soil) – (PO_4^{3-}-P extracted from non-fumigated soil)) / 25.

5.2.8. Soil respiration

Soil respiration was measured once a week with 3 replicates approximately on the plots using the transportable infrared gas analyser CIRAS-1 (PP Systems, Hitchin, UK; Blanke, 1996). The dynamic system consisted of a chamber (100 mm diameter, 150 mm height). For the CO_2 measurements, the steel ring at the bottom of the cylindrical chamber was pushed about 2 cm into the soil. In the chamber, CO_2 enrichment began and was measured for 120 sec or until an increase of about 50 ppm CO_2 was achieved. For calculating the total amount of CO_2 evolved during the experimental period, the CO_2 evolution rate data expressed as mg CO_2-C m^{-2} h^{-1} were taken as representative for the whole day and for the whole period until the next measuring point. Soil temperature was measured concurrently with an attached temperature probe placed 5 cm deep in the soil. Water content was determined each measuring day from samples collected at approximately 0-10 cm depth from each plot.

5.2.9. Statistical analysis

Statistical analyses were carried out using SPSS statistical software (SPSS 15.0). The results presented in tables and figures are arithmetic means and are given on an oven-dry basis (about 24 h at 105 °C for soil samples and about 72 h at 60°C for plant parts). Normality of distribution was tested by the Shapiro-Wilk and Kolmogorov-Smirnov tests. The significance of experimental effects on the microbial properties was tested by a two-way ANOVA, with fertilizer and cropping system as independent factors and sampling day as repeated measures. Experimental effects on yields were tested by a two-way ANOVA,

while experimental effects on yields (in figures) were tested by a crop-specific one-way ANOVA using the LSD test ($P < 0.05$). The relationships between microbial biomass indices, yield and nutrient concentrations in plant tissues were analyzed by Spearman correlation coefficient or by principal component analysis (PCA), using orthotran /varimax rotation to achieve either small or large component loading and an Eigenvalue of 1.0 as the lower limit. PCA was performed using Statistica v7 (Statsoft).

5.3. Results

5.3.1. Plant yield and nutrient concentration

Pea plants started to emerge in all treatments on 20 April (11 DAS). Oats emerged a few days earlier than peas. The number of pea plants varied around 62 m^{-2} ± 5 (standard error) and that of oat plants around 50 ± 5. Peas started to flower on 8 June in the manure treatments and three days later in the other treatments. Percentage of photosynthetically active radiation (PAR) intercepted by the canopy was more than 90% at the two stages of pea development both in sole and intercropped plots (Table 5.2). The intercepted PAR was always significantly higher in the intercropped than in sole pea plots. The same was true for the stand height index. Peas reached the senescence stage on 7 August, corresponding to the late hard dough stage of oats.

Highest pea grain and straw yields of 251 (Fig. 5.2b) and 218 g m^{-2} (Fig. 5.2a), respectively, were found in the control treatment of the sole pea plots. These yields showed a 20 and 14% decrease, respectively, after manure addition and even a 29 and 20% decrease, respectively, after compost addition. Intercropping of peas with oats significantly reduced pea grain and straw yields by 37 and 22%, respectively. However, manure addition had no further depressive effect on pea yields in this case. Intercropping also significantly reduced the harvest index of peas, the number of pods m^{-2} and 100-seed weight (Supplementary Table 1). In 2009, manure and compost addition generally had no significant effects on oat yields. Total grain and straw production in intercropped plots was 90 and 108% higher in comparison with sole pea plots. In 2010, manure and compost addition slightly increased the grain yield of the succeeding winter wheat in comparison with the control treatments after sole pea, while only horse manure increased this yield significantly after intercropped pea (Fig. 5.3). The ANOVA analysis showed significant effects of organic fertilisers ($P < 0.01$) on the wheat. Grain yield of wheat was also significantly ($P < 0.01$) higher after sole peas than after intercropped peas.

Table 5.2: Photosynthetically active radiation (PAR) interception at two sampling dates, equivalent to the pea growth stages BBCH 73 (early pod development) and BBCH 85 (50% of pods ripe); stand height index (plant development at 127 DAS / 75 DAS) in sole peas and in peas intercropped with oats.

	PAR interception (%)		Stand height
	76 DAS	111 DAS	index
Sole peas	94.6	91.8	0.79
Sole peas + manure	95.6	92.6	0.72
Sole peas + compost	95.1	92.0	0.89
Intercropped peas	97.2	96.0	0.97
Intercropped peas + manure	97.8	94.7	0.95
Intercropped peas + compost	97.5	93.7	0.96
Probability values			
Cropping system	<0.01	<0.01	<0.01
Fertilizer	NS	NS	NS
System × fertilizer	NS	NS	NS
CV (±%)	2	3	3

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing.

The main weeds observed were field pennycress (*Thlaspi arvense* L.), common chickweed *(Stellaria media* L.), goosefoot (*Chenopodium album* L.), black bindweed (*Fallopia convolvulus* (L.) A. Löve), wild mustard (*Sinapis arvensis* L.) and German chamomile (*Matricaria recutita* (L.) Rauschert). Weed aboveground biomass production was always significantly lower in the manure treatments at 60 DAS but not at 120 DAS (Table 5.3). Intercropping of peas with oats reduced weed biomass, which comprised on average 42 and 23% of the total biomass in sole pea and intercropped plots, respectively, at 60 DAS, and 45 and 13%, respectively, at 120 DAS. The total above-ground biomass (crops plus weeds) was always significantly lower in manure treatments at 60 DAS but again not at 120 DAS. On average, intercropped plots accumulated 915 g m^{-2} or 25% more plant biomass than sole pea plots.

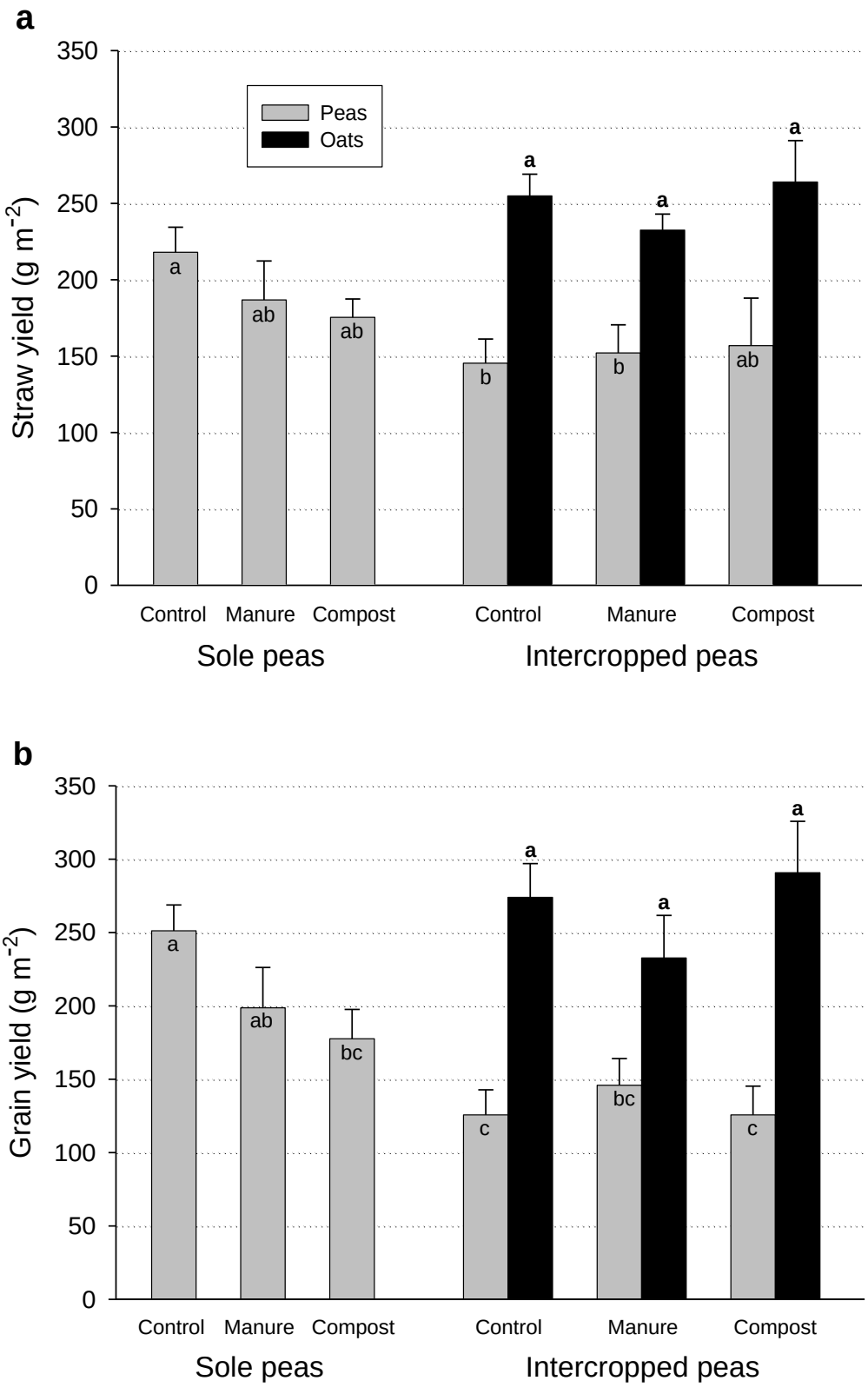

Fig.5.2**:** (a) Straw and (b) grain DW yields at 120 DAS (senescence stage of pea, BBCH 97 and late hard dough stage of oat BBCH 87-89) in sole peas and in peas intercropped with oats; bars = ±1 standard error of mean (n = 4); different letters within a column indicate a significant difference for peas, different letters in bold above the column indicate a significant difference for oats (LSD-test, $P < 0.05$).

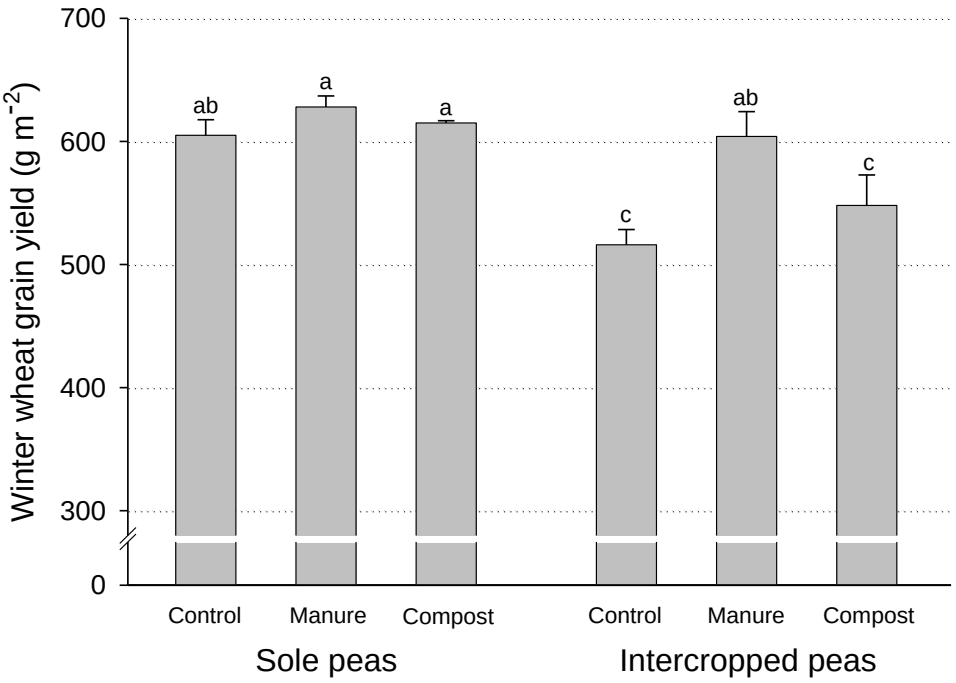

Fig.5.3: Grain yield production of the succeeding crop, winter wheat, as affected by previous treatments; bars = ±1 standard error of mean (n = 4); different letters above the columns indicate a significant difference (LSD-test, $P < 0.05$).

Table 5.3: Yield of weed biomass and total biomass (crop + weed biomass) in sole peas and in peas intercropped with oats at two sampling dates, equivalent to the pea growth stages BBCH 61 (early flowering) and 97 (senescence).

	Weed biomass (g DW m^{-2})		Total biomass (g DW m^{-2})	
	60 DAS	120 DAS	60 DAS	120 DAS
Sole peas	119	301	252	770
Sole peas + manure	53	365	177	750
Sole peas + compost	120	320	261	673
Intercropped peas	58	106	250	906
Intercropped peas + manure	45	133	224	896
Intercropped peas + compost	68	107	283	944
Probability values				
Cropping system	0.01	<0.01	NS	<0.01
Fertilizer	0.01	NS	0.02	NS
System × fertilizer	NS	NS	NS	NS
CV (±%)	37	32	19	13

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing, DW = dry weight.

At 120 DAS, N concentration in pea grain was not affected by cropping system, but was significantly increased by compost and especially manure addition in comparison with the control (Table 5.4). In contrast, N concentration in oat grain was not affected by organic fertilizers. The P concentration was generally increased in the grain of peas and oats by compost and especially manure addition. The P concentration in pea grain was higher in the sole pea than in the intercropped plots. This was also true for the S concentration. However, in contrast to N and P, the S concentration was significantly decreased in pea grain after compost and especially manure addition. This depressive effect of manure on the S concentration was also observed in the oat grain.

Table 5.4: Nutrient concentrations in grain of pea and oat at 120 DAS (senescence stage of pea, BBCH 97 and late hard dough stage of oat BBCH 87-89) in sole peas and in peas intercropped with oats, different letters within a column indicate a significant difference (LSD-test, $P < 0.05$).

Treatment	N	P	S
	(mg g^{-1})		
Peas			
Sole peas	38.1	4.94	2.10
Sole peas + manure	38.9	5.42	1.87
Sole peas + compost	38.8	5.21	1.95
Intercropped peas	37.8	4.74	1.99
Intercropped peas + manure	39.0	5.20	1.78
Intercropped peas + compost	38.6	5.14	1.88
Probability values			
Cropping system	NS	0.07	<0.01
Fertilizer	0.09	0.01	<0.01
System × fertilizer	NS	NS	NS
CV (±%)	2	4	2
Oats			
Intercropped peas	20.5 a	3.56 c	1.39 a
Intercropped peas + manure	20.7 a	3.69 a	1.29 b
Intercropped peas + compost	20.5 a	3.64 b	1.37 ab
CV (±%)	3	2	3

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant.

5.3.2. Microbial root colonization

At 71 DAS, the nodule number of the pea roots was not significantly affected by the organic fertilizers (Table 5.5). In contrast, the nodule number in the peas intercropped with oats was significantly decreased by 32% compared to that of sole peas. At 71 DAS, the ergosterol concentration of pea but also oat roots remained unaffected by any treatment. The same observation was made at 120 DAS. However, at this time, the ergosterol concentration was roughly four times higher than at 71 DAS (Table 5.5). At this time, the roots of both crops were well colonized with arbuscular mycorrhizal fungi (AMF), although the levels of colonization were lower in oat roots (Fig. 5.4). Cropping system had no significant effect on the degree of colonization of pea roots by mycorrhizal hyphae, while application of horse manure and compost resulted in a significant 23% decrease in colonization. Manure and compost addition also reduced the colonization in oat roots by 40 and 20%, respectively.

Table 5.5: Nodule number at late flowering stage of pea (BBCH 69) as well as ergosterol concentrations in root material of pea and oat at two sampling dates, equivalent to the pea growth stages BBCH 69 (late flowering) and BBCH 97 (senescence), in sole peas and in peas intercropped with oats.

	Nodules	Ergosterol (µg g^{-1} DW)			
	(n m^{-2})	Peas		Oats	
	71 DAS	71 DAS	120 DAS	71 DAS	120 DAS
Sole peas	1400	140	462		
Sole peas + manure	1300	122	467		
Sole peas + compost	1700	135	501		
Intercropped peas	1200	114	478	22	81
Intercropped peas + manure	800	167	441	14	74
Intercropped peas + compost	1000	115	497	15	86
Probability values					
Cropping system	<0.01	NS	NS		
Fertilizer	NS	NS	NS	NS	NS
System × fertilizer	NS	NS	NS		
CV (±%)	49	28	21	45	43

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing.

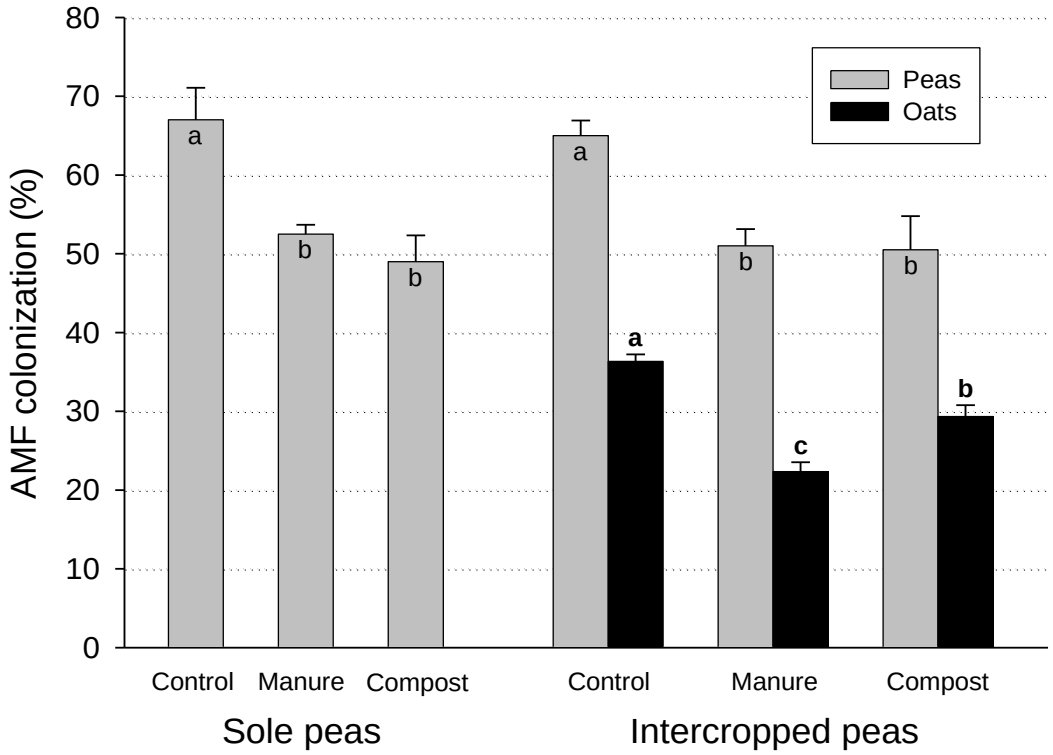

Fig. 5.4: Percentage of mycorrhizal colonization in pea and oat roots at 71 DAS, late flowering of peas (BBCH 69) and late booting stage of oats (BBCH 45/47) in sole peas and in peas intercropped with oats; bars = ±1 standard error of mean (n = 4); different letters within a column indicate a significant difference for pea, different letters in bold above a column indicate a significant difference for oat (LSD test, $P < 0.05$).

5.3.3. Soil microbial biomass indices

Cropping system had no effect on any soil microbial index (Table 5.6). In contrast, manure and compost addition significantly increased the content of microbial biomass C by 85 and 29%, microbial biomass N by 22 and 9%, microbial biomass P by 47 and 11% and ergosterol by 153 and 25%, respectively in comparison with the control treatment. All four soil microbial indices were significantly affected by sampling date. The highest content of microbial biomass C was measured at 120 DAS (Table 5.6), while the highest contents of microbial biomass N and P were measured at 71 DAS. The highest content of ergosterol in the soil was measured at 13 DAS and declined thereafter (Table 5.6).

Table 5.6: Means for main effects of organic fertilizer and cropping system as main factors and sampling days as repeated measures on the contents of microbial biomass C, N, P and ergosterol in soil.

	Microbial biomass			
	C	N	P	Ergosterol
	(μg^{-1} soil)			
Fertilizer treatment				
Control	218	45	19	0.36
Manure	404	55	28	0.91
Compost	281	49	21	0.45
Cropping system				
Sole peas	306	51	23	0.57
Intercropped peas	295	49	23	0.58
Sampling day (DAS)				
13	269	41	22	0.85
71	303	60	25	0.54
120	330	48	22	0.34
Probability values				
Fertilizer	<0.01	<0.01	<0.01	<0.01
Cropping system	NS	NS	NS	NS
Sampling day	<0.01	<0.01	0.02	<0.01
System × fertilizer	NS	NS	NS	NS
System × day	NS	NS	NS	NS
Fertilizer × day	0.01	0.01	0.05	0.04
CV (±%)	11	9	14	32

CV = mean coefficient of variation between replicate plots (n = 4), DAS = days after sowing, NS = not significant.

5.3.4. Principal component analysis and correlation

According to the pea data from the sole and intercropping plots, the first factor considering all treatments explained 35.8% of the variance and was related to soil microbial biomass C, N, and P, but also to the N and P concentrations in the pea grain. A second factor explained another 31.5 % of variance and was related to plant yield and S concentration in the pea grain (Fig. 5.5a).

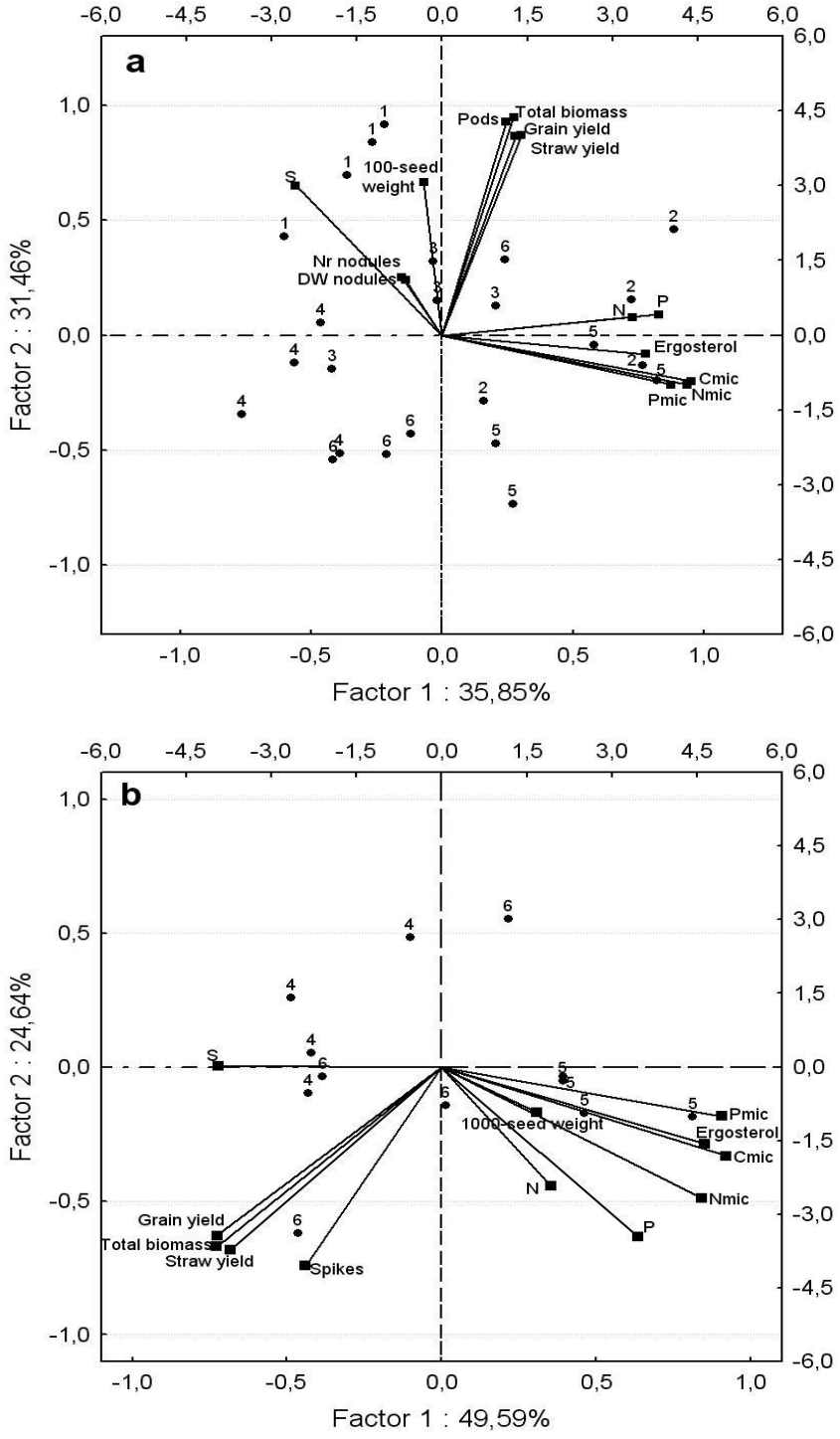

Fig.5.5:Principal component analysis(PCA) for different soil biological and crops yield parameters; (a) for peas and (b) for oats; PCA performed on characteristics, i.e. soil microbial biomass (Cmic, Nmic, Pmic), ergosterol, phosphorus, nitrogen and sulfur in grain(P, N, S), number (No.) and dry weigth (DW) of nodules, straw and grain yield, number of pods / skipes and 100/1000 seed weight; (n=4); (1) sole peas, (2) sole peas+ manure, (3) sole peas + compost, (4) peas intercropped with oats, (5) peas intercropped with oats + manure and (6) peas intercropped with oats + compost.

The number and DW of nodules were the third factor, which explained 13.7% of the variance. On the scatter plot, factors1 and 2 separated the treatments from each other (Fig. 5.5a). Factor 1 had very high positive loadings (> 0.70) from the soil microbial biomass C, N, and P, from N and P concentrations in the pea grain, but was negative loading for the S concentration in the pea grain. Factor 2 had a high positive loading (> 0.65) from plant yield and S concentration in the pea grain.The microbial biomass, ergosterol and N and P concentrations in the pea grain were larger in the horse manure plots, i.e. positive factor1, while these parameters were lower in the non-amended plots. Plant and yield characteristics as well as S concentration were highest in the non-amended sole pea plots, i.e. with highest factor 2 (Fig. 5.5a)

The average contents over the three sampling dates of microbial biomass C, N and P all showed significant positive correlations with N and P concentrations (Spearman correlation coefficient (r) varied between 0.52 – 0.76) in pea grain (Table 5.7), but only with the P concentration in oat grain (r = 0.63 – 0.84). In contrast, significant negative correlations were found between the three microbial biomass indices and the grain S concentrations of both crops (Table 5.7, Fig. 5.5ab). No correlation was observed between pea yield and the amount of microbial biomass (Fig. 5.5a).

5.3.5. Soil respiration

Temporal fluctuations in the CO_2 evolution rates followed the same pattern for all treatments (Fig. 5.6a). CO_2 evolution rates increased gradually with strong fluctuations and peaked from mid-June to mid-July, followed by a continuous decline to the end of the experiment. Cropping system had no effect on CO_2 evolution rates, except for the period from mid-May to mid-June, coinciding with the vegetative growth of crops. In this period, intercropped plots respired on average 12% more CO_2 than sole pea plots. Averaged over the experimental period, mean values for CO_2 evolution rates were 130, 263 and 172 mg C m^{-2} h^{-1} in control, manure and compost treatments, respectively. The total CO_2-C evolution during the period from 22 April to 24 August (124 days) was roughly 4 t CO_2-C ha^{-1} in the control treatment (Fig. 5.6b); manure and compost treatments evolved roughly 100% and 31% more, respectively.

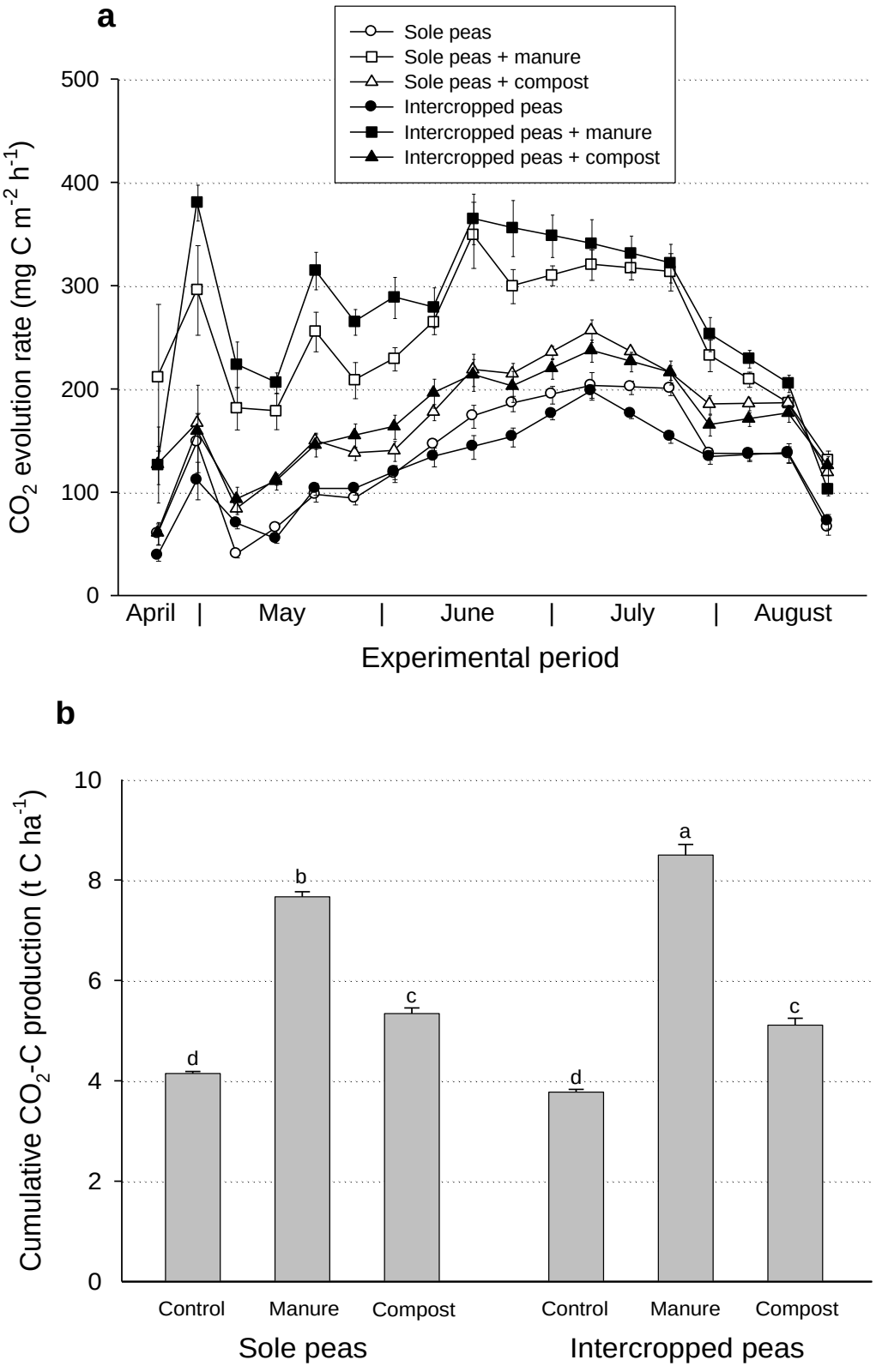

Fig.5.6: (a) CO_2 evolution rates and (b) Cumulative CO_2-C production during the period from 22 April to 24 August 2009(124 days) in sole peas and in peas intercropped with oats; bars = ±1 standard error of mean (n = 12); different letters above a column indicate a significant difference (LSD-test, $P < 0.05$).

5.4. Discussion

5.4.1. Crop yield and nutrient concentrations

Pea grain yields (Fig. 5.2b) in the control treatment of the sole pea plots were similar to those obtained under organic cultivation (Saucke and Ackermann, 2006), but lower than those produced under conventionally grown peas (Neumann et al., 2007, 2009; Ghaley et al., 2005; Hauggaard-Nielsen et al., 2001). Surprisingly, organic fertilizer addition did not enhance the yields of peas and oats in intercropped plots and, moreover, led to a yield reduction in the sole pea plots (Fig. 5.2ab). The added organic fertilizers were rich in easily decomposable components, which may result in competition between soil microorganisms and plants for easily available nutrients, especially N (Kaye and Hart, 1997; Månsson et al., 2009). However, the lack of response cannot be solely attributed to N and P deficiency, as both crops grown in the organic fertilizer treatments exhibited significantly higher P and N concentrations in grain and straw than those grown in the control treatments (Table 5.4). Furthermore, pea, as a legume crop, had a relatively low soil N requirement and a low dependency on soil organic N. As the organic fertilizers, especially horse manure, led to a significant decrease in S concentrations in pea biomass and to some extent also in oat biomass, S deficiency might be one reason for the absence of positive organic fertilizer effects, because legumes have a high S demand (Scherer et al., 2008; Varin et al., 2010), which is, however, less well documented than that of Brassicaceen (Hawkesford et al., 2011).

The absence of positive organic fertilizer effects on crop yields might be caused by the following four factors which caused poor germination, emergence, and early plant growth: (1) inadequate seedbed after distribution of the organic fertilizers by hand and incorporation by a rotary cultivator, (2) formation of large clods especially in manure,(3) water deficiency due to water consumption by decomposition (Prochazkova et al., 2002), and (4) production of phytotoxic substances during further decomposition (Levy and Taylor, 2003; Roy et al., 2010). This is suggested by delayed seedling emergence and the lower yields per plant in comparison with the control treatment (Supplementary Table 2). These problems might be intensified by the generally low plant density, which was below the recommended seed rate for field peas of 80 seeds m^{-2} (Neumann et al., 2007).

In contrast to the year 2009, there was a significant residual effect of the organic fertilizers on the yield of the succeeding winter wheat (Fig. 5.3), suggesting that the

slow release of nutrients from the decomposition of organic fertilizers has significant positive long-term effects (Diacono and Montemurro, 2010). In 2010, the highest yield was recorded with horse manure. This correlates well with the amount of microbial biomass and might be due to microbial decomposition activity in the soil but also to the release of nutrients from the turnover of soil microbial biomass. In 2009, this is also shown by higher CO_2 evolution rates from the soil surface of the manure treatment and by higher N and P concentrations in the crop tissue. The organic components of the compost are apparently more recalcitrant than those of the horse manure.

Intercropping decreased pea nodulation (Table 5.5) and yield components per plant compared with pure stands (Supplementary Table 2). One explanation is the shading of peas by the oat canopy, as indicated by the higher interception of incident PAR in intercropped plots (Table 5.2). Reduced light may affect nodule biomass by restricting photosynthesis of peas and consequently the energy supply to the roots (Ghosh et al., 2006a). Moreover, legumes are less competitive for available inorganic soil N than cereals (Jensen, 1996). Before nodule establishment, the strong early competition for inorganic soil N reduces pea growth and photosynthetic rate, thus intensifying the competitive ability of cereals for light (Ghosh et al., 2006b; Corre-Hellou et al., 2006). The lack of soil N for pea in this early stage may also have a negative effect on further nodulation. Another possible explanation may be the decreased P availability in the presence of oat roots, as indicated by the significantly lower P concentrations in the pea tissue of the intercropped plots in comparison with sole pea plots (Table 5.4). The present results are in agreement with Ghosh (2004) and Jensen (1996) but contradict others, suggesting that intercropping facilitates nodule development and N_2 fixation of legumes (Izaurralde et al., 1992; Li et al., 2009). Another explanation for the generally lower pea yield in the intercropped plots is the intense belowground competition from the cereal partner, which often exhibits more extensive root systems and higher initial growth rates than legumes (Jensen, 1996). However, the total yield of the intercrop plots was significantly greater than that of the sole pea plots (Table 5.3), indicating a better utilization of growth resources, i.e. water, nutrients, and radiation energy (Hauggaard-Nielsen et al., 2008).

In the present experiment, the weed biomass showed a strong negative correlation with the total yields of peas and oats ($r = -0.81$, $P < 0.01$, $n = 24$), indicating that weeds were a significant competitor for growth resources and caused reductions in crop yields (Table 5.3). Field peas are generally considered to be poorly competitive against weeds

(McDonald, 2003; Deveikyte et al., 2009). This is due to (1) the slow initial growth rate of pea seedlings, (2) the low plant density and (3) the weak weed suppression ability of the semi-leafless peas (McDonald, 2003; Saucke and Ackermann, 2006). Our results provide further evidence for the importance of intercropping as a method for controlling weeds, especially in organic farming systems (Hauggaard-Nielsen et al., 2008).

5.4.2. AMF colonization and ergosterol content

Organic fertilizer addition significantly reduced colonization of pea and oat roots in the field by native AMF (Fig. 5.4), as observed by Ellis et al. (1992) and Tarkalson et al. (1998) for other species, although contrary results have also been reported (Muthukumar and Udaiyan, 2000; Labidi et al., 2007). One explanation for the present results would be an increased microbial competition in the rhizosphere (Tarkalson et al., 1998). Another possible explanation would be that the addition of available nutrients, especially P, decreases the dependency of plants on AMF (Ellis et al., 1992). In the present experiment, AMF colonization of pea roots was higher than that of oat roots. It is known that plant species differ in their dependency on AMF (van der Heijden et al., 1998). It has been shown that nitrogen-fixing legumes are more mycorrhizal dependent and generally have higher levels of root colonization than grasses to supply extra phosphorus required for nodule formation (Azcón et al., 1991; Plenchette and Morel, 1996; Scheublin et al., 2007).

In arable and grassland soils, the fungal cell-membrane component ergosterol is an important and highly specific indicator for the presence of fungal biomass (Joergensen and Wichern, 2008). The concentration of ergosterol was roughly 6 times higher in pea than in oat roots (Table 5.5), suggesting that saprotrophic fungi are strongly affected by plant species. Also Appuhn and Joergensen (2006) and Frey et al. (1992) measured higher ergosterol concentrations in legume (*Vicia sativa* L., *Trifolium alexandrinum* L.) than in cereal roots (*Triticum aestivum* L., *Zea mays* L.).The quality and higher quantity of rhizodeposition derived from peas in comparison with oat (Wichern et al., 2007) may explain the difference. Ergosterol concentrations in root material of both crops dramatically increased during maturation in the field, reaching values up to 500 µg g^{-1} DW, suggesting that saprotrophic fungi colonize senescent or newly dead root parts. Medina et al. (2003) measured ergosterol concentrations of up to 350 µg g^{-1} DW in *Medicago sativa* roots at 84 days after planting in pots. Colonization of plant roots by AMF has been shown to improve plant growth and productivity, especially legumes

(Arias et al., 1991; Stancheva et al. 2006). However no significant correlation was found between native AMF and pea yield (r = 0.12) or oat (r = 0.35) in this study.

5.4.3. Microbial biomass indices

In contrast to the crop yield, the addition of the two organic fertilizers resulted in immediate significant increases in all microbial biomass indices (Table 5.6). These increases are most likely due to the microbial biomass contained in the organic fertilizers (García-Gil et al., 2000, Rasul et al., 2008). During the vegetative growth of crops, the continuous supply of rhizodeposition contributed to the maintenance and further growth of the soil microbial biomass (Wichern et al., 2007). A noteworthy result of this study is the positive correlations observed between microbial biomass indices C, N and P in the soil and the concentrations of N and P in plant material of both species (Fig. 5.5, Table 5.7). Our results are consistent with those of Saini et al. (2004), who found a significant relationship ($P < 0.01$) of microbial biomass C, N and P with N and P uptake of the two crops sorghum and chickpea at three different stages of crop growth. Under greenhouse conditions, Chen et al. (2000) found that ryegrass P concentration was more closely correlated with microbial biomass P than with any other chemical index of soil P availability. The significant negative correlation between the three microbial biomass indices and grain S concentrations suggests that microbial S immobilization might be caused by the organic fertilizer addition, leading to the absence of positive effects on crop yield, however positive correlation was found between the grain S concentration and grain yield and total biomass in both species (r = 0.46 – 0.50).

Table 5.7: Spearman correlation coefficients between nutrient concentrations in grain of peas and oats and the mean contents of microbial biomass C, N, P over the growing season (n = 24 for peas and 12 for oats).

	Pea grain			Oat grain		
	N	P	S	N	P	S
Microbial biomass C	0.63**	0.76**	-0.67**	0.35	0.73**	-0.76**
Microbial biomass N	0.64**	0.72**	-0.65**	0.47	0.84**	-0.57*
Microbial biomass P	0.52**	0.64**	-0.60**	0.37	0.63*	-0.62*

* $P < 0.05$, ** $P < 0.01$

5.4.4. Soil respiration

Under field conditions, CO_2 evolution is the sum of microbial and root respiration (Jensen et al., 1996). However, measurement of soil-surface CO_2 emissions in situ can be used to estimate the mineralization of applied organic amendments (Rochette et al., 2006; Terhoeven-Urselmans et al., 2009). During the vegetative stage, intercropped pea plots evolved significantly more CO_2 than sole pea plots (Fig. 5.6a). This can probably be explained by the higher amount of rhizodeposition in this treatment, fuelling the turnover of the microbial biomass (Wichern et al., 2007). However, differences in water availability and soil temperature may additionally contribute to the differences in the CO_2 evolution rates observed (Curiel Juste et al., 2007; Ding et al., 2007).

Assuming that the addition of organic fertilizers did not affect the decomposition of native soil organic matter, approximately 40% of the manure C and 24% of the compost C were mineralised to CO_2 during this period. These C mineralization rates are similar to those observed for different organic amendments under field conditions (Lee et al., 2007; Rochette et al., 2006). Manure and compost addition increased CO_2 concentrations above the soil surface by 100 and 33%, respectively, in comparison with the control treatments, following the same trend of the microbial biomass, which indicates a large contribution of microbial respiration to the total soil CO_2 evolution (Kazunori and Oba, 1994; Lee et al., 2007). The absence of any plant response to the elevated CO_2 concentration is in line with the results of Ding et al. (2007), who observed no significant correlation of cumulative CO_2 production to wheat biomass in the field, but contrasts their results for maize in the same experiment, suggesting a crop-specific response.

5.5. Conclusions

Short term application of horse manure and compost greatly stimulated soil microbial biomass and CO_2 production, but failed to stimulate productivity of the current crops. Consequently, no correlation existed between any of the yield parameters and microbial biomass indices. In contrast, the close relationships between grain N and P concentrations and microbial biomass C, N and P suggest that the soil microbial biomass can be used as an indicator of nutrient availability to plants. Significant positive residual effects of organic fertilizer, especially horse manure, were observed on the grain yield of the succeeding wheat. Mycorrhizal colonization and ergosterol content

in roots differed significantly between the two crops, without any effects on yield. Intercropping is an important tool for controlling weeds on pea plots under organic farming conditions. The addition of organic manures one day before sowing, poor seedling emergence and microbial S immobilization appear to be the reasons for the absence of positive organic fertilizer effects on crop yield.

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6. Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions

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ABSTRACT

In a field experiment, peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) were grown as sole crops and intercrops, fertilized with horse manure and yard-waste compost derived from shrub and garden cuttings at 10 t C ha^{-1} each. The objectives were to compare the effects of these organic fertilizer and cropping system in organic farming on (a) yield of peas and oats, grown as the sole crop or intercropped, as well as N_2 fixation and photosynthetic rates, (b) the yield of wheat as a succeeding crop, (c) microbial biomass indices in soil and roots, and (d) microbial activity estimated by the CO_2 evolution rate in the field and the amount of organic fertilizers, recovered as particulate organic matter (POM). In general, organic fertilizer application improved nodule dry weight (DW), photosynthetic rates, N_2 fixation, and N accumulation of peas as well as N concentration in oat grain. Averaged across fertilizer treatments, pea/oat intercropping significantly decreased nodule DW, N_2 fixation and photosynthetic rate of peas by 14, 17, and 12%, respectively, and significantly increased the photosynthetic rate of oats by 20%. However, the land equivalent ratio (LER) of intercropped peas and oats exceeded 1.0, indicating a yield advantage over sole cropping. Soil microbial biomass was positively correlated with pea dry matter yields both in sole and intercropped systems. Organic fertilizers increased the contents of microbial biomass C,

N, P, and fungal ergosterol in soil and CO_2 production, whereas the cropping system had no effects on these microbial indices. According to the organic fertilizer recovered as POM, 70% (manure) and 64% (compost) of added C were decomposed, but only 39% (manure) and 13% (compost) could be attributed to CO_2-C during a 101-day period. This indicated that horse manure was more readily available to soil microorganisms than compost, leading to increased grain yields of the succeeding winter wheat.
Keywords: Horse manure, Yard-waste compost, Microbial biomass, CO_2 evolution, Particulate organic matter, Intercropping, Photosynthesis

6.1. Introduction

Peas (*Pisum sativum* L.) are usually grown in rotation or intercropped with cereal crops, particularly with spring barley (*Hordeum vulgare* L.; Jensen, 1996), spring wheat (*Triticum aestivum* L.; Ghaley et al., 2005) and oats (*Avena sativa* L.; Neumann et al., 2007). Intercropping is the simultaneous cultivation of two or more crop species on the same area of land (Willey, 1979). Intercropping of legumes with cereals is of particular importance in organic agriculture to increase yield stability and decrease weed pressure and diseases (Hauggaard-Nielsen et al., 2009; Corre-Hellou et al., 2011). Studies on legume and cereal intercropping have found significant yield advantages of intercropping compared to sole cropping, with a land equivalent ratio (LER) of up to 1.34 (Andersen et al., 2004; Ghaley et al., 2005), especially if little or no fertilizer N was applied (Ghaley et al., 2005). This advantage is mainly due to greater use efficiency of plant growth resources, i.e. water, nutrients and radiation energy (Hauggaard-Nielsen et al., 2001).

The ability of legumes to fix atmospheric N_2 is important in organic farming, where this process is the major source of N input (Berry et al., 2002). Most studies show that nodulation and N_2 fixation of legumes may be improved by intercropping (Neumann et al., 2007), because the cereal component is more competitive for soil N, forcing the legume crop to rely on N_2 fixation (Andersen et al., 2004). In contrast, Ghosh et al. (2006) reported that intercropping significantly reduced photosynthesis, nodulation and N_2 fixation of the legume crop due to shading by the cereal component. In general, cereals are considered to be the dominant crop component in pea/cereal intercropping

systems (Jensen 1996; Ghaley et al., 2005; Neumann et al., 2007), because the cereal is more competitive for above- and belowground growth resources. Few intercropping trials have been carried out under organic farming conditions (Hauggaard-Nielsen et al., 2009). At the same time, little attention has been paid to belowground interaction and soil microbial properties of intercropping systems, which may account for their yield advantage (Song et al., 2007), as suggested by the positive correlations between soil microbial biomass and crop yields (Saini et al., 2004; Khan and Joergensen, 2006).

The use of organic fertilizers is a major component of organic farming practices (Berner et al., 2008). Organic manures can provide the essential plant nutrients and enhance crop productivity, but also leave a beneficial residual effect on succeeding crops (Ghosh et al., 2004). Various kinds of organic materials such as animal manures, sewage sludge and crop residues are applied to soil to improve soil organic matter content and consequently, the physical, chemical and biological properties of the soil (Debosz et al., 2002)). However, little attention has been paid to some types of manure such as yard-waste compost and especially horse manure, which contain large amounts of woody debris and bedding straw, respectively, and have a wide C/N ratio. The decomposition of such carbon rich organic manures may cause temporary N-immobilization and consequently restrict crop productivity (Thomsen and Kjellerup, 1997; Berry et al., 2002). Decomposition has been measured based on the recovery of organic substrates added, using litterbags (Beare et al., 2002) or the recovery of unused substrate as particulate organic matter (POM) (Magid et al., 1997). The POM method has been successfully used in the laboratory (Magid and Kjaergaard, 2001; Jannoura et al., 2012) but also in the field (Magid et al., 1997).

The in situ decomposition of organic fertilizers can also be estimated by quantifying the amount of CO_2-C evolved from the soil surface caused by amendment (Rochette et al., 2006; Terhoeven-Urselmans et al., 2009). Consequently, increased CO_2 concentrations in the atmosphere may result in more efficient photosynthesis and growth, especially of legumes (Rogers et al., 2006; Fischinger et al., 2010). This response of photosynthesis to elevated CO_2 has been repeatedly observed under greenhouse conditions (Jin et al., 2009) as well as under field conditions (Rogers et al., 2006). Photosynthesis is one of the major physiological processes influencing the yield and quality of many crops (Peng et al., 1991; Liu et al., 2004). Positive relationships between photosynthetic rates and crop yields have been reported for many crops (Efthimiadou et al., 2009), while other research groups found little (Buttery et al., 1981)

or no correlation (Chango and McVetty, 2001). Under field conditions, N status and source as well as plant density and intercropping may affect plant photosynthesis (Makoi and Ndakidemi, 201; Zhou et al., 2011). Both pot and field experiments have shown that organic manure can generally increase photosynthesis (Liu et al., 2004; Antolín et al., 2010).

In the present field experiment, horse manure and yard-waste compost derived from shrub and tree clippings was supplied at 10 t ha^{-1} C but different N amounts to field peas or oats, either as a sole crop or intercropped. The underlying hypotheses were: (1) C rich organic fertilizers have generally beneficial effects on pea productivity as sole crop due to the positive effects on N_2 fixation and photosynthetic rate. (2) Organic fertilizers also have beneficial effects on the productivity of intercropped peas and oats estimated by an increase in the LER ratio. (3) The shading effect of the cereal component will reduce the photoynthesis, nodulation and N fixation of intercropped pea. (4) Manure has stronger effects than compost due to the better C availability to soil microorganisms. The specific objectives were to compare the effects of organic fertilizer and cropping system in organic farming on (a) the yield of peas and oats, grown as the sole crop or intercropped, as well as N_2 fixation and photosynthetic rates, (b) the yield of wheat as a succeeding crop, (c) microbial biomass indices in soil and roots, and (d) microbial activity estimated by the CO_2 evolution rate in the field and the amount of organic fertilizers, recovered as POM.

6.2. Materials and methods

6.2.1. Site, soil, and organic fertilizers

The field experiment was carried out from April to August 2010 for the target crops and from October 2010 to August 2011 for the succeeding wheat crop at the experimental farm of the University of Kassel, Frankenhausen in northern Hessia (51° 24' N, 9° 25' E, and 248 m ASL). The long-term mean annual rainfall is 698 mm. In 2010, the annual precipitation was 441 mm and thus 37% lower, with 97 mm falling in August (Fig. 6.1a). The long-term mean annual temperature is 8.5 °C. In 2010, the mean air temperature was 8 °C and thus 0.5 °C lower. Over the experimental period, soil temperature varied between 10.3 and 23.7 °C, with a mean of 16.7 °C (data not shown). Temporal variation in soil moisture at 0-10 cm depth responded to changes in the

temporal pattern of rainfall and varied widely between 4.5% (w/w) in July and 24.5% in June (Fig. 6.1b).

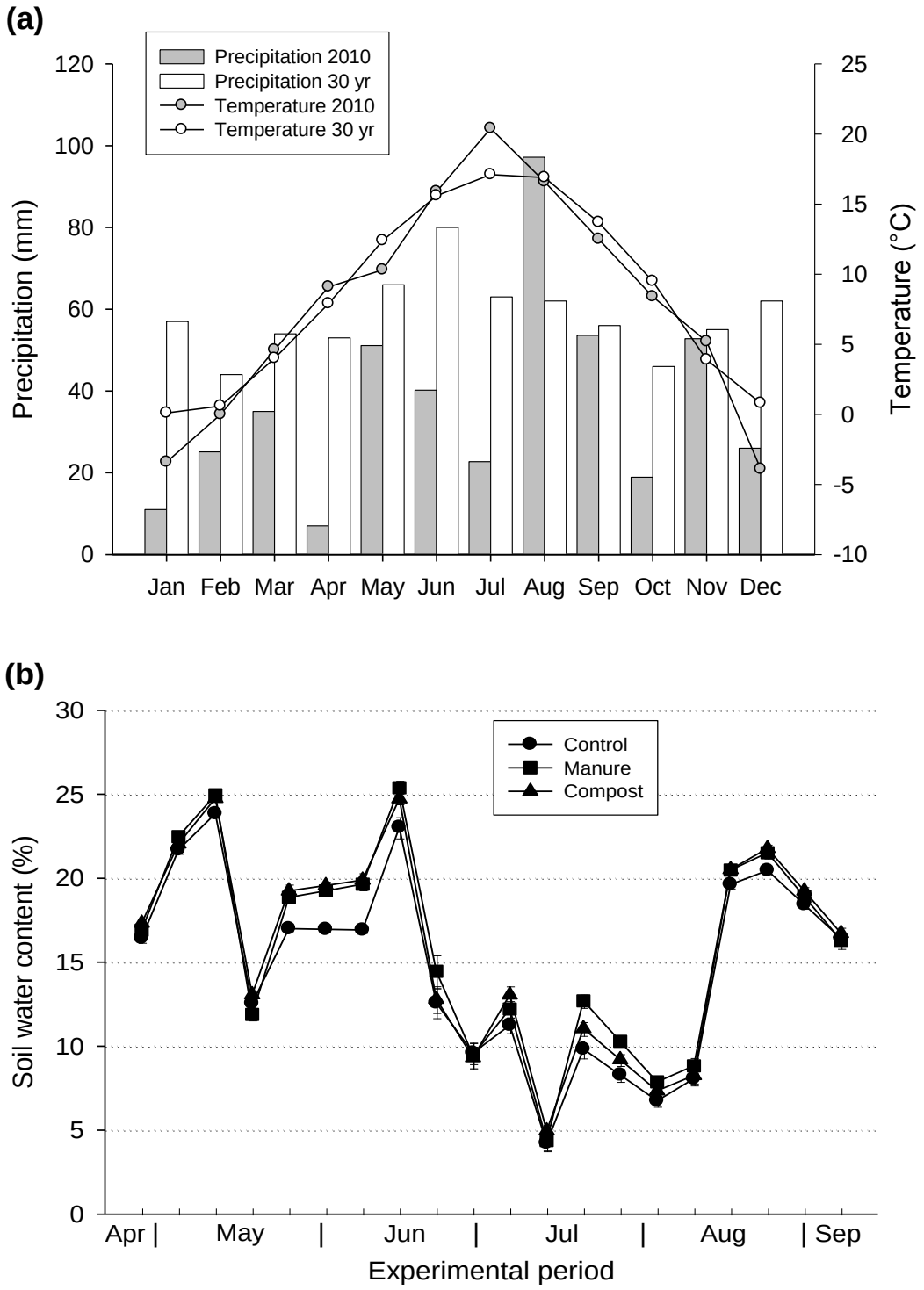

Fig.6.1: (a) Mean monthly precipitation (bars) and temperature (line) for 2010 as compared to the long-term (1960-1990) at the study site. (b) Soil water content at 0-10 cm depth in the experimental plots during the period from 27 April to 7 September 2010 as affected by three fertilizer treatments: no fertilizer (control), horse manure and compost. The data were presented across the three cropping systems, because cropping system had no effect on soil moisture at any of the measuring dates. Vertical bars indicate ± one standard error of mean (n = 36).

The site was ploughed to a depth of 25 cm on 15 October 2009, followed by a shallow seedbed preparation on 14 April 2010 using a rotary harrow. Fertilizers were applied and distributed by hand on 19 April, then incorporated to a depth of 18 cm by rotary cultivator. The crops were sown on 23 April 2010. Peas were sown at 80 seeds m^{-2} both in sole and intercropped plots, while oats were sown at 300 kernels m^{-2} in sole plots and at 60 kernels m^{-2} in the intercropped plots. Intercropped peas and oats were sown in the same row. Plant populations were determined by counting the number of seedlings in three randomly selected 1 m lengths of rows in each plot.The crops were cultivated according to organic agricultural practice with no use of herbicides and with mechanical weeding three times during emergence and leaf development stages of peas. After sowing and during the early stages of leaf development, the experimental area was covered with nylon nets to avoid bird damage. The preceding crops were a mixture of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne L.*) in 2008 and potatoes (*Solanum tuberosum* L.) in 2009. For determining the residual effect of organic fertilizer applied on the yield of the succeeding winter wheat, the experimental plots were ploughed again on 13 October 2010, followed by a shallow seedbed preparation using a rotary harrow. Winter wheat (*Triticum aestivum* L., cv. Achat) was sown on 14 October at a density of 300 seeds m^{-2} and a row distance of 18.75 cm.

The soil was classified as Haplic Luvisol (Quintern et al., 2006) and contained 17% clay, 81% silt, 2% sand, 1.3% total C and 0.15% total N, its pH (H_2O) was 7 and it had a water holding capacity of 49%. The soil was sampled three times during the growing season (Table 6.1) on 12 May (19 DAS), on 29 June (67 DAS) and on 2 August 2010 (101 DAS). From each plot, three samples were collected with a soil corer (7 cm diameter) to a depth of 20 cm at three random points between the rows. Cores from each plot were bulked, mixed thoroughly and a representative sample of 1000 g was taken. A subsample (400 g) of this soil was passed through a 2-mm sieve, adjusted to 50% water holding capacity and stored in polyethylene bags at 4 °C until soil biological analysis was carried out.

The organic manures used were fresh horse manure, mixed with stall beddings (wheat straw), and rotted yard-waste compost (shredded shrubs and tree clippings; 3 months old). Manure and compost were given to supply equivalent amounts of 10 t C ha^{-1} to the respective treatments. The horse manure had a pH of 8.0 and contained 422.6 mg C, 10.9 mg N, 2.0 mg P, 16.6 and mg K g^{-1} DW. The compost had a pH of 5.7 and contained 271.1 mg C, 9.8 mg N, 0.51 mg P, 4.5 mg K g^{-1} DW. Potassium sulphate and

soft rock phosphate were added to the control and compost plots to supply amounts of K and P equivalent to those in the manure plots.

6.2.2. Experimental design

Semi-leafless field peas (*Pisum sativum* L. var. Santana KWS, Einbeck) and oats (*Avena sativa* L. var Dominik) were grown as sole crops and as intercrops under three fertilizer treatments: (1) control without fertilizers, (2) horse manure and (3) yard-waste compost. The trial was conducted in a split plot design with four replicates, using the fertilizer treatments as main plots and the three cropping treatments ((a) sole peas, (b) sole oats, and (c) peas and oats intercropped) as subplots (6 × 4.5 m). The subplots were separated from each other by a walking path of 0.5 m width. Each subplot was divided into three 6 × 1.5 m sub-subplots: one for final yield determination, where the plants were left undisturbed until harvest, and the other two for sampling and measurements. Sub-subplots consisted of seven rows with a row distance of 18.75 cm.

Crop development was recorded at approximately weekly intervals according to the BBCH code (Meier, 1997). Plant stand heights were measured from the soil surface to the highest point in the canopy at three places in each plot after flowering of peas (80 DAS) and at maturity of peas (115 DAS). The stand height index was calculated as stand height at maturity divided by stand height at flowering (Sauermann, 2007). The crops were harvested twice during the growing season (Table 6.1), once at flowering stage (62 DAS) and twice at physiological maturity (94 DAS).

At flowering stage, above-ground plant biomass was determined by cutting the plants at soil level from an area of 1 m² of each plot. The material was separated into component crops, dried at 60°C for 72 h and weighed. In addition, all plants in a sampling area of 33 cm row length × 37.5 cm width (two rows) of both sole and intercropped pea plots were harvested. Above-ground plant parts were cut at soil level. Intercrops were separated into peas and oats.The plants were counted and placed in separate plastic bags for determination of leaf area. Then the soil was excavated to a depth of approximately 25 cm. After initial soil removal, the roots of each plant were placed in plastic bags with some water.

Table 6.1: Dates of soil and plant sampling, and photosynthesis measurements, days after sowing (DAS), BBCH code and phonological growth stages of pea (*Pisum sativum* L.) and oat (*Avena sativa* L.).

Description	Date	DAS	Pea		Oat	
			BBCH	Stage	BBCH	Stage
Soil sampling						
(1) at emergence	12 May	19	10-11	Early leaf development	11	Early leaf development
(2) at flowering	29 June	67	69	Late flowering	58	End of heading/pre-anthesis
(3) at harvest	2 August	101	89	Fully ripe	87	Hard dough stage
Plant sampling						
(1) at flowering	24 June	62	62	Early flowering	45	Late boot
(2) at harvest	26 July	94	89	Fully ripe	87	Hard dough stage
Photosynthesis measurements						
(1)	14-15 June	52	19-35	Leaf development/stem elongation	32	Early stem elongation
(2)	29-30 June	67	69	Late flowering	58	End of heading/pre-anthesis
(3)	16-17 July	84	81	Beginning of ripening	75-77	Milky ripe

BBCH: abbreviation of growth stages of plant species derived from Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (Meier, 1997).

Oats in sole crop plots were harvested by cutting 6 plants per plot at the soil level and their roots were excavated to a depth of about 20 cm. Sampled plants were kept in plastic bags. Plastic bags containing plant parts were put in an ice box and transferred immediately to the laboratory. The samples were stored at 4 ºC until analysis. For assessment of nodulation in pea plants, the root system of each plant was carefully washed on a sieve under running water to remove adhered soil, and then nodules were detached from the roots and their numbers recorded. The dry weights of nodules were determined after drying at 50 °C for 72 h. Samples of roots were taken for determination of mycorrhizal colonization and ergosterol as a biomarker for saprotrophic fungi.

At final harvest, above-ground plant biomass was also harvested from 1 m^2. The material was separated into component crops, dried at 60 °C for 72 h and weighed. After threshing, grain yields of peas and oats were determined. Dried plant material was ground and analysed for total C and total N using a Vario Max CN analyser (Elementar, Hanau, Germany). Five additional randomly selected plants (for each species) from each plot were cut at the soil surface for determining the yield components per plant. Roots were sampled with a soil corer (7 cm diameter × 15 cm depth) for ergosterol determination. The succeeding winter wheat was harvested on 19 August 2010. The samples were dried at 60°C for 72 h and weighed for determination of dry weight production.

6.2.3. Photosynthesis and leaf area index

Photosynthetic rates of peas and oats were measured on 6 randomly selected plants per plot at three stages of pea development, at 52 DAS, at 67 DAS and at 84 DAS (Table 6.1). All readings were taken between the hours of 10 a.m. and 2 p.m. on two sequential days each measuring date. The photosynthesis of oats at 52 DAS was not measured because of the bad weather conditions. Measurements were made on the second fully expanded leaves below the top of peas and on the leaves directly under the flag leaf of oats using a portable gas exchange system (LI-6400, LiCor, Lincoln, NE, USA). The photosynthetic rate was recorded after stabilization of the value. To obtain the mean photosynthetic rate during the measuring period (Peng et al., 1991), rates were averaged across the three measurement dates for peas and the two measurement dates for oats.

At flowering (67 DAS), three harvested plants for each species per plot were selected and separated into leaves and stems. The leaf area of each plant was measured with a leaf area meter (Delta T device, UK) and total leaf area per plant was calculated by adding the area of individual leaves. The leaves of each plant were dried at 60°C for 72h and weighed. The specific leaf area was obtained by dividing total leaf area per plant by the dry mass of leaves ($cm^2\ g^{-1}$). The leaf area index (LAI) was calculated by multiplying the average leaf area per plant ($m^2\ plant^{-1}$) by the total number of plants per m^2.

6.2.4. Mycorrhizal colonization and ergosterol

Fresh root material of peas and oats (1.5 g) was cut into 1 cm length and cleared in 10% KOH for 60 min at 65°C for peas and at 90°C for oats. After rinsing with tap water, the samples were acidified with 2 M HCl for 20 min, and then stained with 0.1% trypan blue in 90% lactic acid for 20 min at 65°C for peas and at 90°C for oats (Phillips and Hayman, 1970; Kleikamp and Joergensen, 2006). Subsequently the roots were distained with lactic acid and stored in a solution containing lactic acid, glycerol and water (1:1:1 v/v/v) until examination. The percentage of root length colonized was determined with the gridline intersection method (Giovannetti and Mosse, 1980) using a binocular microscope at × 40 magnification. Ergosterol was extracted and measured according to Djajakirana et al. (1996). Moist samples of 0.5 g root material and 2 g soil were extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rpm. Ergosterol was measured by reversed-phase HPLC analysis with a mobile phase of 100% methanol and a resolution of detection of 282 nm.

6.2.5. N_2 fixation

The amount of symbiotically fixed nitrogen at final harvest was calculated by the extended difference method according to Stuelpnagel (1982) for sole cropped peas (Eq. 1) and according to Karpenstein-Machan and Stuelpnagel (2000) for intercropped peas (Eq.2).

$$N - Fix_{PS} = (N_{PS} - N_{OS}) + (soil\ N_{PS} - soil\ N_{OS}) \quad [1]$$

$$N - Fix_{POI} = (N_{POI} - N_{OS}) + (soil\ N_{POI} - soil\ N_{OS}) \quad [2]$$

where N_{PS}, N_{OS} and N_{POI} are the amounts of nitrogen in the above-ground dry matter of sole peas, sole oats and intercrops, respectively; soil N_{PS}, soil N_{OS} and soil N_{POI} are the inorganic N in soil at 0-60 cm depth after peas, oats and intercrops, respectively. Inorganic N of soil was extracted with 0.0125 M $CaCl_2$ at a ratio of 1:4 and the content of NO_3-N was analysed colourimetrically using a continuous flow analyser (CFA) (Evolution2, Alliance Instruments, Germany).

6.2.6. Soil microbial biomass

Microbial biomass C and N were estimated using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Moist soil of 20 g was spilt into two portions of 10 g. One portion was fumigated for 24 h with ethanol-free $CHCl_3$. After its removal, the soil was extracted with 40 ml of 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 rpm and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The non-fumigated portion was extracted in the same way. Organic C in the K_2SO_4 extracts was measured as CO_2 by infrared absorption after combustion at 850°C using a Dimatoc 100 analyser (Dimatec, Essen, Germany). Microbial biomass C was E_C / k_{EC}, where E_C = (organic C extracted from fumigated soil) - (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion. Microbial biomass N was E_N / k_{EN}, where E_N= (total N extracted from fumigated soil) - (total N extracted from non-fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985).

Soil microbial biomass P was measured by fumigation extraction (Brookes et al., 1982). Three portions equivalent to 2.5 g oven-dry soil were each extracted with 50 ml of 0.5 M $NaHCO_3$ (pH 8.5) by 30 min horizontal shaking at 150 rpm, centrifuged for 10 min at 2000x*g* and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). The first portion was fumigated (see above), the second portion was non-fumigated and the third portion was used for estimating the recovery of 25 μg P g^{-1} soil added as KH_2PO_4 to the extractant. Phosphorus was analysed by an ammonium molybdate-ascorbic acid method as described by Joergensen et al. (1995). Microbial biomass P was E_P / k_{EP} / recovery, where E_P = (PO_4^{3-}-P extracted from fumigated soil) - (PO_4^{3-}-P extracted from non-fumigated soil) and k_{EP} = 0.40 (Brookes et al., 1982).

6.2.7. Soil respiration

Soil respiration was measured once a week with 3 replicates approximately on the plots using a transportable infrared gas analyser CIRAS-1 (PP Systems, Hitchin, UK, Blanke, 1996). The dynamic system consisted of a chamber (100 mm diameter, 150 mm height). For the CO_2 measurements, the steel ring at the bottom of the cylindrical chamber was pushed about 2 cm into soil. In the chamber, CO_2 enrichment began and was measured for 120 s or until an increase of about 50 ppm CO_2 was achieved. For calculating the total amount of CO_2 evolved during the experimental period, the CO_2 evolution rate data expressed as mg CO_2-C m^{-2} h^{-1} were taken as representative for the whole day and for the whole period until the next measuring point (Terhoeven-Urselmans et al., 2009). Soil temperature was measured concurrently with an attached temperature probe placed 5 cm deep in the soil. Water content was determined each measuring day from samples collected at 0-10 cm depth with a manual probe from each plot, using the difference in weight before and after drying of a soil sample at 105°C.

6.2.8. Particulate organic matter (POM)

Particulate organic matter was determined by size separation through wet sieving according to Magid and Kjaergaard (2001) as described by Muhammad et al. (2006). Moist soil of 400 g was dispersed in 500 ml of 5% NaCl, shaken by hand and allowed to stand for 45 min. Then the samples were poured gradually onto two sieves of 2 mm and 0.4 mm mesh size and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve until the water passing through the sieve became clear. The material retained on the sieve was transferred into a bucket. Tap water was added, the bucket was swirled and organic material was separated repeatedly from the mineral material by flotation-decantation, until organic particles were no longer visible in the mineral fraction. Then, the mineral fraction was discarded. The remaining two particulate organic matter POM fractions 0.4-2 and > 2 mm were transferred to crucibles, dried at 60 °C, weighed and ground for analysis. Total C and total N were determined using a Vario Max CN analyser (Elementar, Hanau, Germany).

6.2.9. Calculation and statistical analysis

The land equivalent ratio (LER), which is defined as the relative land area required as sole crops to produce the same yields achieved in intercropping (Willey, 1979), was calculated for both total DM production using the following equation:

$$LER = \frac{Y_{P-IC}}{Y_{P-SC}} + \frac{Y_{O-IC}}{Y_{O-SC}} \quad [3]$$

where $Y_{P\text{-}IC}$ and $Y_{P\text{-}SC}$ are the yields of intercropped and sole peas and $Y_{O\text{-}IC}$ and $Y_{O\text{-}SC}$ are the yields of intercropped and sole oats, respectively. LER values >1 indicate intercropping advantages in terms of improved use of environmental resources. When LER <1 resources are used more efficiently by sole crops than intercrops.

Assuming that the addition of organic fertilizers did not affect the decomposition of soil organic matter, the percentage of manure or compost C recovered as POM-C in the two fractions 0.4-2 and >2 mm was calculated as follows:

$$C_{recovered} = \left[\frac{(POM\text{-}C_{amended} - POM\text{-}C_{control})}{C_{added}} \right] \times 100 \quad [4]$$

where $POM\text{-}C_{amended}$ and $POM\text{-}C_{control}$ represent average POM-C recovered in the amended treatments and in the respective control treatment, respectively. C_{added} represents the amount of organic C initially added with manure or compost. A similar equation was used to calculate the percentages of manure or compost N recovered in the two POM-N fractions.

The percentage of manure or compost C mineralised as CO_2-C in a certain period after application was calculated as follows:

$$C_{mineralized} = \left[\frac{CO_2\text{-}C_{amended} - CO_2\text{-}C_{control}}{C_{added}} \right] \times 100 \quad [5]$$

where $CO_2\text{-}C_{amended}$ and $CO_2\text{-}C_{control}$ represent average cumulative CO_2-C evolved in the amended treatments and in the respective unfertilised control treatment, and C_{added} represents the amount of initially added organic C.

Statistical analyses were carried out using SPSS statistical software (SPSS 15.0). The results presented in tables and figures are arithmetic means and are given on an oven-dry basis (about 24 h at 105 °C for soil samples and about 72 h at 60°C for plant

parts). The significance of experimental effects on the microbial properties was tested by a two-way ANOVA with fertilizer and cropping system as independent factors and sampling day as repeated measures. Experimental effects on plant data in tables were tested by a two-way ANOVA, while experimental effects on plant data in figures were tested by a crop-specific one-way ANOVA using the LSD test ($P < 0.05$). The Spearman correlation coefficient was used to determine correlations between different variables

6.3. Results

6.3.1. Yields and photosynthetic rates

During the growing season, sole and intercropped plants of both species showed similar phenological development stages according to the BBCH code. However, during maturation, sole cropped oats reached the ripe stage faster than intercropped oats. Actual plant densities of peas and oats were slightly higher than the target densities, both in sole and intercropped plots, with the exception of oats provided with manure, which had considerably lower densities both in sole and intercropped systems.

In sole cropped peas, the lowest straw (Fig. 6.2a) and grain (Fig. 6.2b) yields of 225 and 335 g m^{-2} were recorded in the control treatment. Manure and compost application significantly increased straw yield by 20 and 28%, respectively, and grain yield by 16 and 14%, respectively. In contrast, when intercropped, only manure application significantly increased straw and grain yields of peas by 45 and 75%, respectively, in comparison with the control treatment. Averaged across fertilizer treatments, intercropping decreased pea straw and grain yield by 42 and 53%, respectively, as well as number of pods per m^2 and 100 seed weight (see also Supplementary Table 3). Straw and grain yields of oats with compost application were similar to the control treatment for sole and intercropped oats, while manure application significantly decreased these yields by 11 and 30% for sole and intercropped oats, respectively. LER values of intercropped plots did not differ significantly between fertilizer treatments and varied around 1.2 and 1.1, when calculated for total DW production at flowering and harvest, respectively. However, horse manure application significantly increased the partial LER of peas to 0.64 (+ 0.17) and decreased that of oats to 0.50 (- 0.13) in comparison with the control.

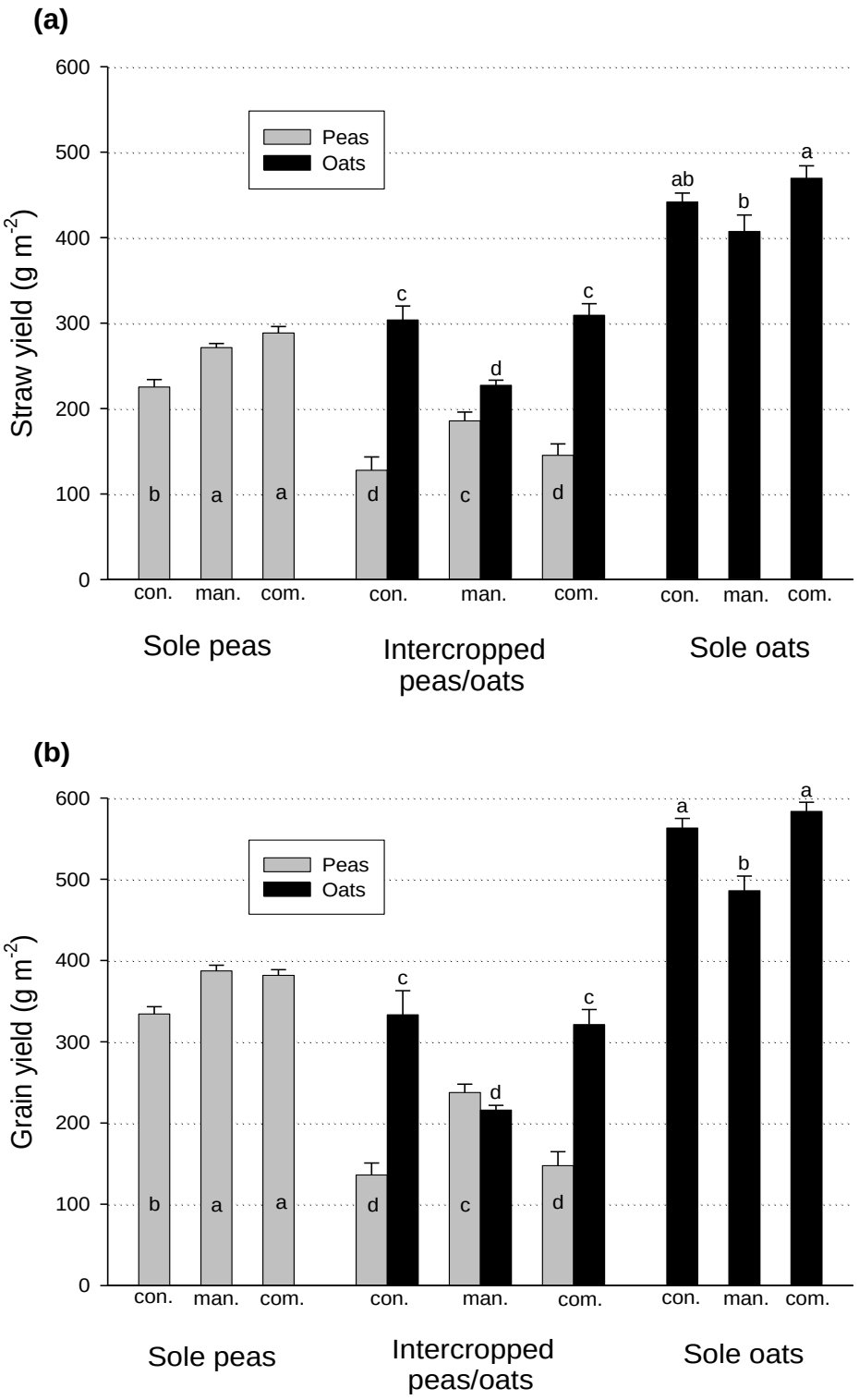

Fig.6.2: (a) Straw and (b) grain DM yields of pea and oat at 94 DAS in three cropping systems with three fertilizer treatments: control without fertilizer (con.), horse manure (man.) and compost (com.). Error bars indicate ± one standard error of mean (n=4), different letters within columns indicate a significant difference for pea, different letters in bold above the columns indicate a significant difference for oat according to the LSD test ($P < 0.05$).

At 52 DAS, the photosynthetic rate of pea plants was not affected by cropping system and was on average 12 µmol m^{-2} s^{-1} in control plots (Fig. 6.3a). Manure and compost application increased photosynthetic rates of peas on average by 7 and 25%, respectively. In contrast, at the last two measuring dates, i.e. 67 and 84 DAS, photosynthetic rates of both species were only affected by the cropping system but not by the fertilizer treatment. An exception was sole cropped peas, for which higher rates were recorded in the compost treatment (Fig. 6.3b and c). Averaged across fertilizer treatments, intercropping decreased the photosynthetic rate of peas by 9 and 23% and increased that of oats by 17 and 20% at 67 and 84 DAS, respectively. The highest photosynthetic rates were generally recorded at 67 DAS, followed by 84 DAS. Manure and compost application significantly increased mean photosynthetic rates of sole cropped peas by 4 and 12%, respectively, and intercropped peas by 5 and 7%, respectively, in comparison with the respective control treatments. In contrast, the mean photosynthetic rate of oats was not significantly affected by fertilizer treatment. Intercropping decreased the mean photosynthetic rate of peas by an average of 12% but increased that of oats by 20% in comparison with the sole cropped plots. For peas, there were significant positive correlations between mean photosynthetic rate and yield in grain, straw and total aboveground biomass ($n = 24$; $r = 0.69$ - 0.77, all $P < 0.01$).

In 2011, manure and compost addition significantly increased the grain yield of winter wheat in the three cropping systems (Fig. 6.4). Averaged across fertilizer treatments, the higher wheat grain yield of 656 g m^{-2} was recorded in treatments in which wheat crop was preceded by sole pea, followed by a pea/oat intercropping system (592 g m^{-2}). The lowest grain yield of 519 g m^{-2} was recorded when wheat was preceded by sole oat. The ANOVA analysis also showed significant effects of organic fertilizers and cropping system ($P < 0.01$) on the wheat grain yield.

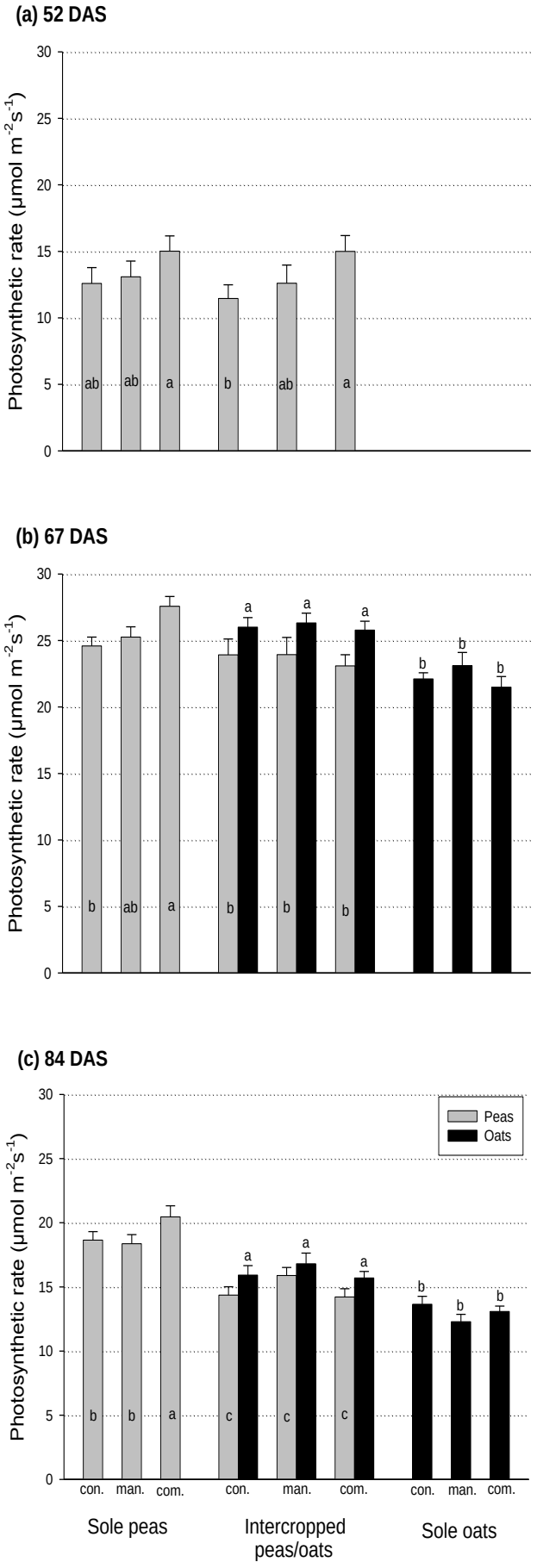

Fig.6.3: Photosynthetic rates of the leaves of pea and oat grown under three cropping systems with three fertilizer treatments: control without fertilizer (con.), horse manure (man.) and compost (com.). Measurements were made at 52 (a), 67 (b) and 84 (c) DAS for pea and at 67 (b) and 84 (c) DAS for oat. Error bars indicate ± one standard error of mean (n = 24), different letters within columns indicate a significant difference for pea, different letters in bold above the columns indicate a significant difference for oat according to the LSD test ($P < 0.05$).

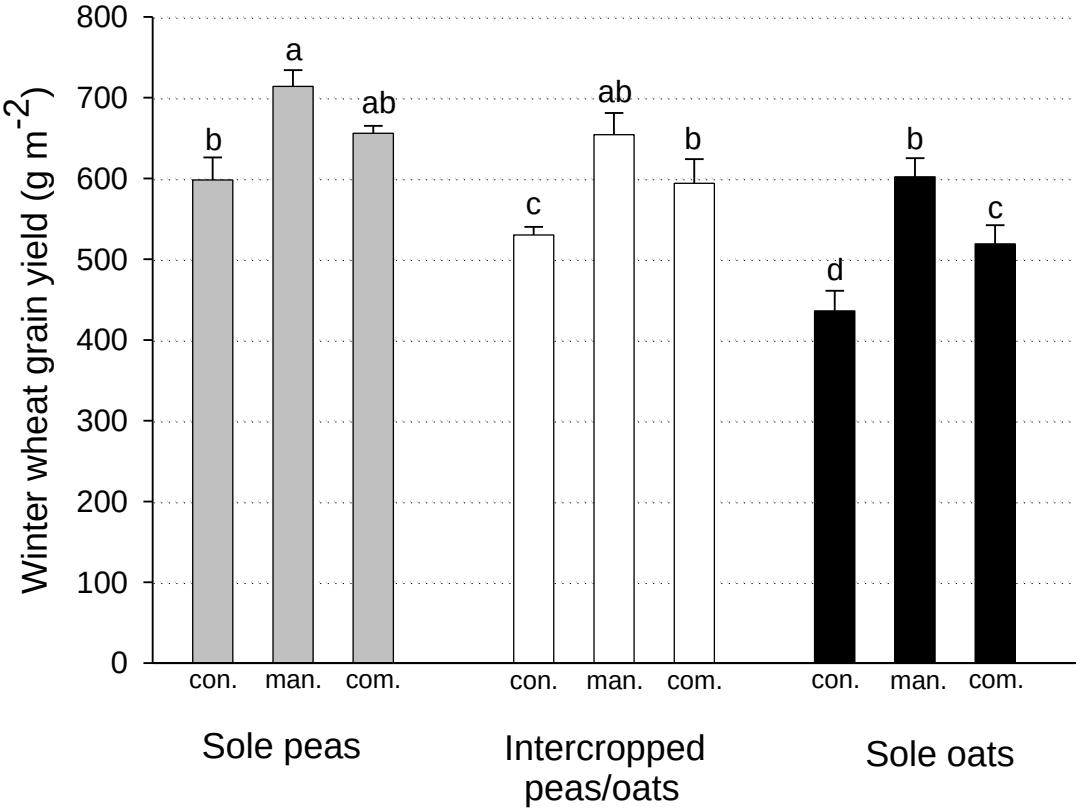

Fig.6.4: Grain yield production of the succeeding crop, winter wheat, as affected by previous treatments; bars = ±1 standard error of mean (n = 4); different letters above the columns indicate a significant difference (LSD-test, P < 0.05)

6.3.2. Nodulation, N_2 fixation, and N uptake

In sole cropped peas, manure and compost application significantly increased nodule DW (Fig.6.5) by 40 and 20%, respectively, in comparison with the control treatment. In intercropped peas, only manure increased nodule DW by 24%. Averaged across fertilizer treatments, intercropping decreased nodule DW by 14% in comparison with sole cropped peas. The amount of N_2 fixed by the sole cropped peas was 46 kg N ha^{-1} in the control treatment (Fig. 6.5), which was increased by 155 and 67% in the manure and compost treatments, respectively. Intercropped peas fixed 51 kg N ha^{-1} in the control treatment, which was increased by 60 and 33% in the manure and compost treatments, respectively. Averaged across fertilizer treatments, intercropping significantly decreased N_2 fixation by 17%. A significant relationship was observed between nodule DW and the amount of N_2 fixed (r = 0.58; $P < 0.01$).

The N concentration in grain and straw of peas (Table 6.2) was similar in the control and compost treatments but significantly lower in the manure treatment, independently of the cropping system. In contrast, the N concentration in grain and straw of oats responded

significantly to organic fertilizers in the order manure > compost > control. On average, intercropping significantly increased grain and straw N concentration by 5 and 6%, respectively, in peas, and by 16 and 81%, respectively, in oats. The N uptake of sole cropped peas was 158 kg ha^{-1} in the control treatment (Table 6.2).

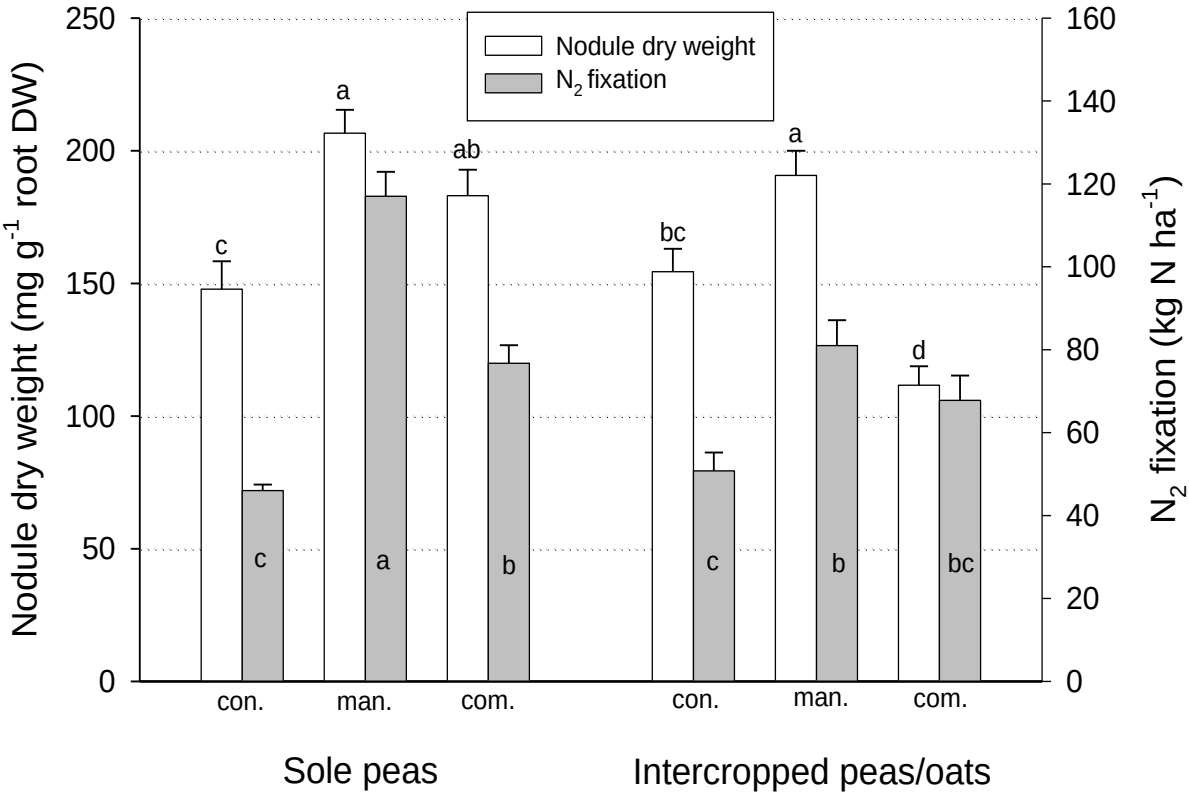

Fig.6.5: Nodule dry weight at 62 DAS (early flowering stage, BBCH 62) as well as the amount of N_2 fixed at 94 DAS (fully ripe stage BBCH 89) of sole and intercropped pea with three fertilizer treatments: control without fertilizer (con.), horse manure (man.) and compost (com.). Error bars indicate ± one standard error of mean (n= 29–45 for nodule dry weight and 4 for N_2 fixation), different letters above the columns indicate a significant difference for nodule dry weight, different letters in bold within columns indicate a significant difference for N_2 fixation according to the LSD test ($P < 0.05$).

Table 6.2: N Concentrations in grain and straw as well as aboveground N accumulation of pea and oat grown under three cropping systems: sole pea, peas intercropped with oats and sole oats with three fertilizer treatments: control without fertilizer, horse manure and compost at 94 DAS.

Treatment	N concentration (mg g^{-1})				Aboveground N (kg ha^{-1})				Total
	Pea		Oat		Pea		Oat		
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	
Sole peas	40.1	10.5			134	24			158
Sole peas+ manure	39.0	9.8			151	27			178
Sole peas + compost	40.6	10.8			155	31			186
Intercropped peas/oats	42.4	11.8	22.3	7.3	58	15	75	22	170
Intercropped peas/oats + manure	40.8	10.0	23.3	9.6	97	19	50	22	188
Intercropped peas/oats + compost	42.0	11.3	22.4	7.7	62	16	72	24	174
Sole oats			18.6	4.0			104	18	122
Sole oats + manure			20.4	5.4			99	22	121
Sole oats + compost			19.5	4.2			114	20	134
Probability values									
Cropping system	<0,01	0.03	<0,01	0.01	<0.01	<0.01	<0.01	0.05	<0.01
Fertilizer	<0.01	0.01	<0.01	<0.01	<0.01	0.01	<0.01	NS	0.01
System x fertilizer	NS	NS	NS	NS	0.01	0.05	0.07	NS	0.02
CV (±%)	1	6	2	11	11	13	4	14	5

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing

In the manure and compost treatments, peas significantly accumulated 20 and 28 kg more N ha^{-1}, respectively, than peas in the control treatment. The N uptake of sole cropped oats was similar, with 121 kg ha^{-1} in control and manure treatments, but 13 kg ha^{-1} higher in the compost treatment. In the intercropped plots, the N uptake of peas and oats was on average 177 kg N ha^{-1} without any fertilizer effect. The proportion of pea N in intercrops was on average 44% in the control and compost treatments and 62% in the manure treatment. For peas, significant positive correlations were observed between mean photosynthetic rate and N accumulation in grain, straw, and total aboveground biomass (n = 24; r = 0.71 - 0.77, all $P < 0.01$), as well as nodule DW (n = 24; r = 0.56, $P < 0.01$). For oats, N concentration in grain and straw was significantly correlated with the mean photosynthetic rate (n = 24; r = 0.73 and 0.75, all $P < 0.01$).

6.3.3. Microbial root colonization

Roots of peas and oats were well colonized with indigenous mycorrhizal fungi, but the percentage colonization was higher for peas (Fig. 6.6). Intercropping led to a reduction in mycorrhizal colonization only for peas. Irrespective of cropping system, organic fertilizers significantly decreased root colonization by 18% for peas and 31% for oats. Ergosterol concentrations in pea roots were on average 85 µg g^{-1} DW at 62 DAS and 245 µg g^{-1} DW at 94 DAS, without any effect of cropping system or fertilizer treatment (Table 6.3). Ergosterol concentrations in oat roots were on average 7 µg g^{-1} DW at 62 DAS and 34 µg g^{-1} DW at 94 DAS, i.e. markedly lower than in pea roots.

6.3.4. Microbial biomass indices

Manure application significantly increased the contents of microbial biomass C and N by an average of 52%, microbial biomass P by 67% and especially ergosterol by 131% in comparison with the control treatments (Table 6.4). The corresponding increases due to compost application were 23, 60 and 33%, respectively. The microbial biomass C/P ratio was significantly lower in the organic fertilizer treatments than in the control treatment, while the inverse effect was observed for the ergosterol to microbial biomass C ratio. The microbial biomass C/N ratio remained unaffected by the organic fertilizers. The cropping system had no effect on the soil microbial biomass indices analysed, except ergosterol, which was higher in intercropped than in sole cropped plots. This resulted in a significantly higher ergosterol to microbial biomass C ratio. The

highest contents of microbial biomass C, N and ergosterol as well as the highest microbial biomass C/N and C/P ratios were measured at 101 DAS. Significant correlations were observed between the mean (average of the growing season) microbial biomass C, N and P contents and yield in grain, straw and total aboveground biomass for sole and intercropped peas (n = 12; r = 0.56 - 0.87, all $P < 0.01$). There were also significant correlations between the mean microbial biomass C, N and P, and the N concentrations in grain and straw for sole and intercropped oats (n = 12; r = 0.56 - 0.87, all $P < 0.01$).

Table 6.3: Ergosterol concentration in roots of pea and oat at 62 and 94 DAS under three cropping systems: sole peas, peas intercropped with oats and sole oats, with three fertilizer treatments: control without fertilizer, horse manure and compost.

Treatment	Ergosterol in root material (µg g^{-1} dw)			
	62 DAS		94 DAS	
	Pea	Oat	Pea	Oat
Sole peas	101		233	
Sole peas + manure	94		254	
Sole peas + compost	78		280	
Intercropped peas/oats	69	8	258	41
Intercropped peas/oats + manure	94	6	210	23
Intercropped peas/oats + compost	78	5	248	40
Sole oats		10		39
Sole oats + manure		8		26
Sole oats + compost		6		34
Probability values				
Cropping system	0.07	0.05	NS	NS
Fertilizer	NS	0.06	NS	0.02
System x fertilizer	0.05	NS	NS	NS
CV (±%)	16	29	15	28

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing

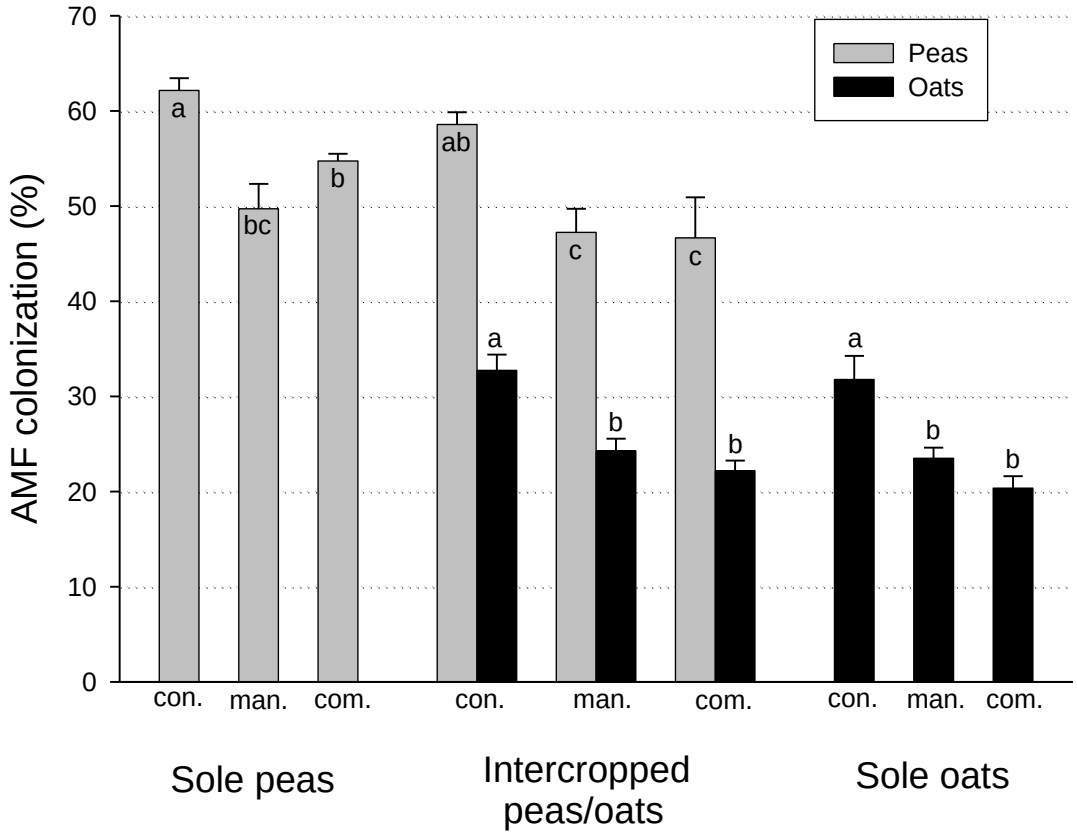

Fig.6.6: Percentage of mycorrhizal colonization in pea and oat roots at 62 DAS (early flowering stage of pea (BBCH 62) and the late booting stage of oat (BBCH 45)), in three cropping systems with three fertilizer treatments: control without fertilizer (con.), horse manure (man.) and compost (com.). Error bars indicate ± one standard error of mean (n=4), different letters within columns indicate a significant difference for pea, different letters in bold above the columns indicate a significant difference for oat according to the LSD test ($P < 0.05$).

6.3.5. CO_2 evolution and particulate organic matter (POM)

The cropping system used had no effect on CO_2 evolution rates. Therefore, the rates were averaged across the three cropping systems (Fig. 6.7a). CO_2 evolution increased gradually from 27 April and peaked in mid-June, followed by a continuous decline to the end of the experiment. The several peaks observed in CO_2 evolution rates coincided with higher soil moisture (Fig. 6.1b). Significant differences in CO_2 evolution rates between fertilizer treatments were observed at each measuring day ($P < 0.01$, one-way ANOVA with LSD test). The CO_2 evolution rates during the experimental period ranged from 45 - 330 mg C m^{-2} h^{-1} in the control treatments, from 115 - 710 mg C m^{-2} h^{-1} in the manure treatments, and from 80 – 480 mg C m^{-2} h^{-1} in the compost treatments (Fig. 6.7a). Averaging across the three cropping systems, 4.8, 9.4, and 6.4 t CO_2-C ha^{-1} 133 day^{-1} were evolved from the control, manure, and compost treatments, respectively

(Fig. 6.7b). At 101 DAS, 39 and 13% of manure and compost C, respectively, were recovered as CO_2-C, assuming that the organic fertilizers did not affect the decomposition of soil organic C (Fig. 6.8).

Table 6.4**:** Means for main effects of organic fertilizers and cropping systems as main factors and sampling days as repeated measures on the contents of microbial biomass C, N, P and ergosterol and on the ratios microbial C/N, microbial C/P and ergosterol/microbial biomass C in soil.

Treatment	Microbial biomass					Ergosterol	Ergosterol/ microbial biomass C
	C	N	P	C/N	C/P		
	($\mu g\ g^{-1}$ soil)					($\mu g\ g^{-1}$ soil)	(%)
Fertilizer							
Control	291	51	15	5.7	20.6	0.51	0.18
Manure	443	78	25	5.6	17.7	1.18	0.28
Compost	351	64	24	5.6	15.8	0.68	0.20
Cropping system							
Sole peas	357	63	21	5.7	18.0	0.77	0.22
Intercropped peas/oats	365	65	22	5.6	17.8	0.84	0.23
Sole oats	371	65	21	5.7	18.4	0.75	0.20
Sampling day							
19 DAS	290	53	21	5.6	14.3	0.72	0.25
67 DAS	380	69	23	5.5	17.6	0.70	0.19
101 DAS	423	71	20	5.9	22.3	0.97	0.23
Probability values							
Fertilizer	<0.01	<0.01	<0.01	NS	<0.01	<0.01	<0.01
Cropping system	NS	0.07	NS	NS	NS	0.01	0.01
Sampling day	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
System × fertilizer	NS	NS	NS	NS	0.08	NS	NS
System × day	0.01	0.01	NS	0.01	NS	NS	0.01
Fertilizer × day	0.01	NS	0.01	0.01	NS	0.03	0.03
CV (±%)	7	5	15	4	16	12	13

CV = pooled coefficient of variation between replicate plots (n = 4), NS = not significant

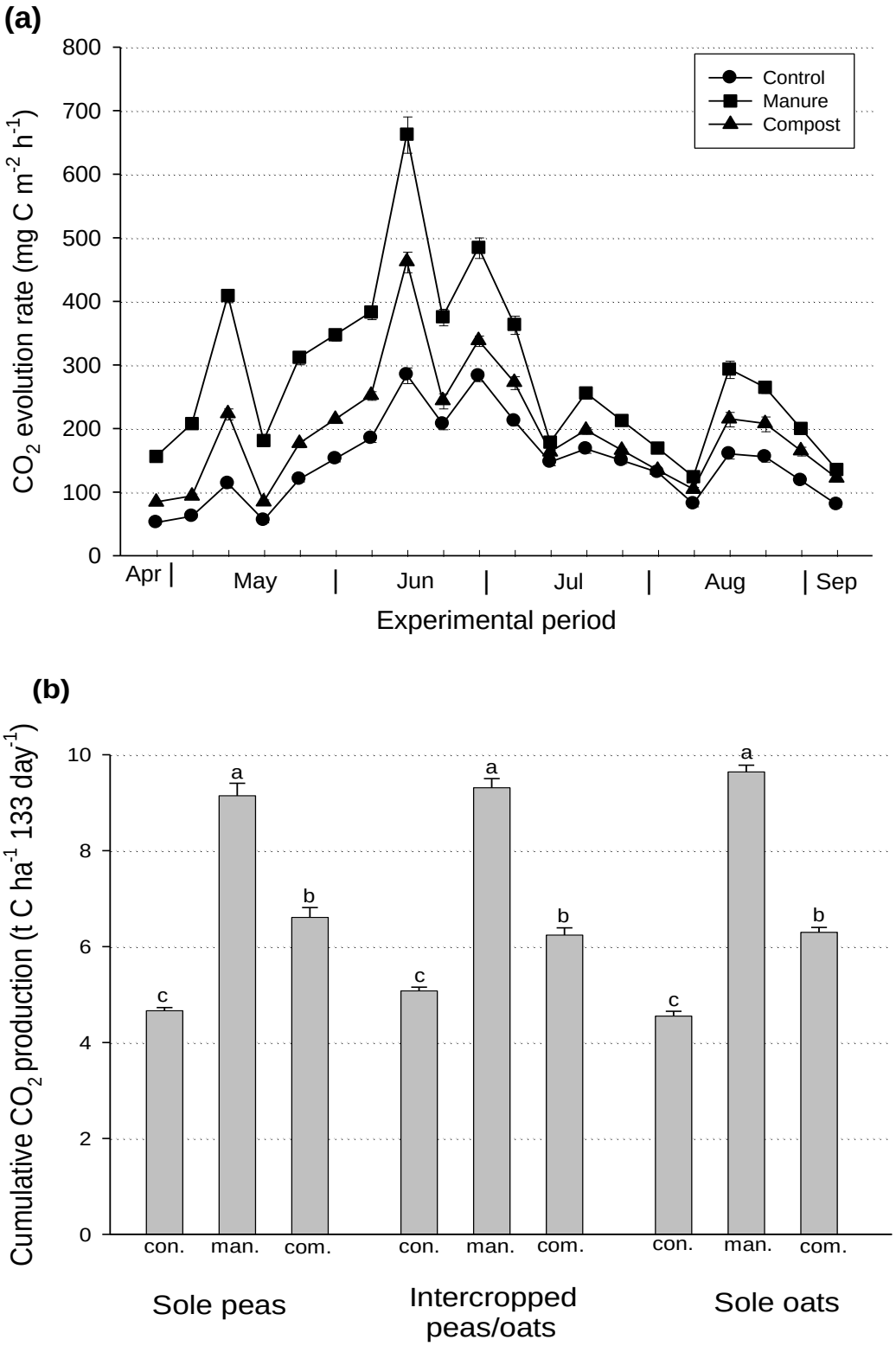

Fig.6.7: (a) CO_2 evolution rates from the surface of the plots during the period from 27 April to 7 September 2010 as affected by three fertilizer treatments: control without fertilizer, horse manure and compost. The data were presented across the three cropping systems, because cropping system had no effect on CO_2 evolution rate at any of the measuring dates. Vertical bars indicate ± one standard error of mean (n = 36). (b) Cumulative CO_2-C production during the period from 27 April to 7 September 2010 (133 days) as affected by three cropping systems with three fertilizer treatments: control without fertilizer (con.), horse manure (man.) and compost (com.). Error bars indicate ±

one standard error of mean (n = 12), different letters above the columns indicate a significant difference according to the LSD test (P < 0.05).

The cropping system also had no significant effect on the amounts of the two POM fractions (0.4-2 mm; >2 mm) recovered (Supplementary Tables 6ab). Therefore, the data were averaged across the three cropping systems (Fig. 6.8). At each sampling day, less total POM-C was recovered in manure plots compared to compost plots. At 101 DAS, 30 and 36% of manure C and compost C, respectively, were recovered as total POM-C. In contrast, at each sampling date, similar amounts of total POM-N were recovered from the manure and compost treatments. On average 75, 66 and 52% of total N added as organic fertilizer were recovered at 19, 67 and 101 DAS, respectively.

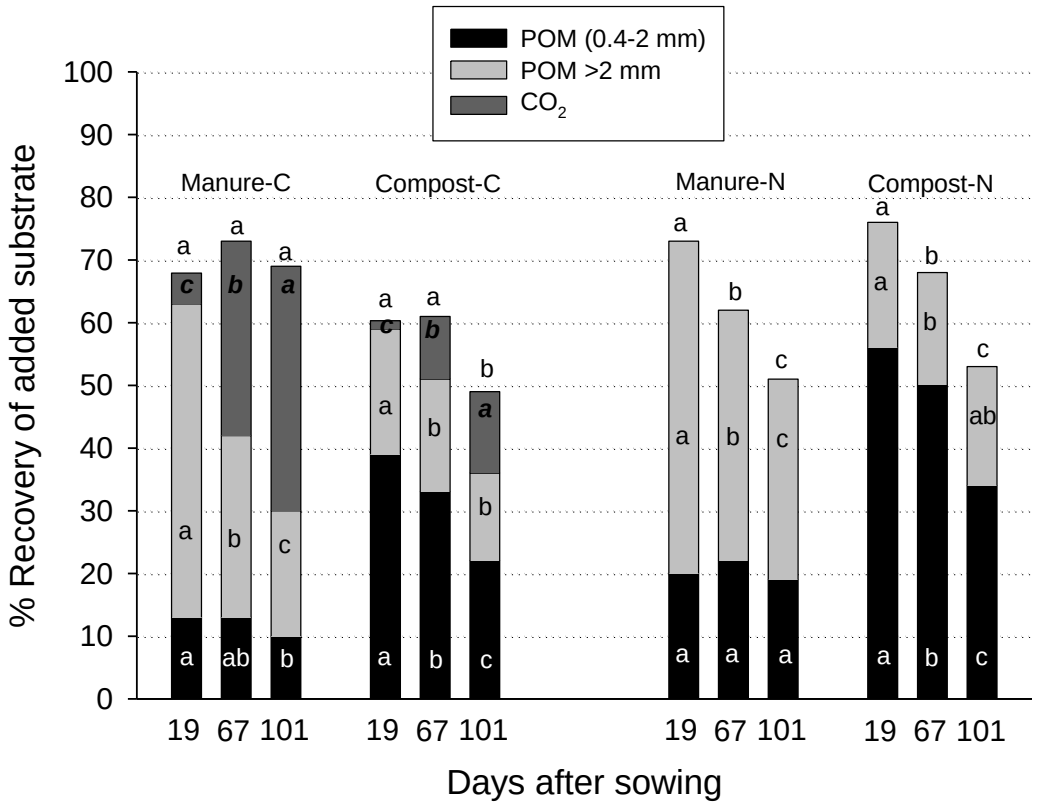

Fig.6.8**:** Manure or compost C recovered as POM in the fractions 0.4–2 and >2 mm and as CO_2–C, manure or compost N recovered as POM in the fractions 0.4–2 and >2 mm at three sampling dates: 19, 67 and 101 days after sowing. The data were presented across the three cropping systems because cropping system had no effect on the amount of C or N recovered at any of the measuring dates. Different letters in white within bars indicate a significant difference for the POM fraction (0.4-2 mm), different letters in bars indicate a significant difference for the POM fraction (>2 mm), different letters in bold within the bars indicate a significant difference for CO2-C, and different letters above the bars indicate a significant difference for the total recovery of substrate according to the LSD test (P < 0.05). The statistical analysis was performed separately for manure-C or N and compost-C or N.

6.4. Discussion

6.4.1. Yields and photosynthetic rates

The application of manure and compost increased the yield of peas but not that of oats. Aboveground yields of peas and oats were in the range reported for organic field experiments across Western Europe (Neumann et al., 2007; Hauggaard-Nielsen et al., 2009). Highly significant relationships were observed between pea yields and N uptake, nodulation, N_2 fixation and mean photosynthetic rate, indicating the contribution of these indices to the higher crop productivity. Under field conditions, significant and positive correlations between leaf photosynthesis and crop yields have been reported for other crops, such as soybean (Ghosh et al., 2006), sorghum (Peng et al., 1991), and sweet corn (Efthimiadou et al., 2009). Manure and compost increased the average photosynthetic rate of pea plants. Similar results have also been observed in pot (Antolín et al., 2010) and field experiments (Liu et al., 2004), adding other organic fertilizers to legumes.

One of the main benefits of intercropping is an increase in yield per unit land area, expressed as LER values (Hauggaard-Nielsen et al., 2009). Averaged across fertilizer treatments, intercropping of pea with oat significantly decreased nodule DW, N_2 fixation, photosynthetic rate and pea yields. This can be attributed to lower light availability due to shading by the relatively tall oat plants, greatly restricting photosynthesis and energy supply to nodules (Nambiar et al., 1983). In addition, this shading effect also reduced growth attributes such as leaf area, leaf area index, and dry matter accumulation per plant (Supplementary Tables 4 and 5). Reductions in nodule mass and photosynthetic rate of the legume component has also been observed in a soybean/sorghum intercropping system (Ghosh et al., 2006). At the vegetative stage (52 DAS), intercropping did not reduce the photosynthetic rate of peas, because the shorter height of the oat plants did not lead to effective shading.

In contrast to peas, the higher plant density of sole cropped oats reduced light energy and, consequently, led to significantly lower photosynthetic rates than in intercropped oats (Makoi and Ndakidemi, 2011). Furthermore, intra-specific competition for other plant growth factors such as mineral nutrients, especially N, and water may be another reason; this was indicated by the far lower N concentration in grain and straw of the sole compared to the intercropped oats (Zhao et al., 2005). When intercropped with peas, oats had significantly higher grain and straw N concentrations

as a result of their greater competitive ability to take up soil inorganic N sources (Jensen, 1996; Hauggaard-Nielsen et al., 2001). The direct transfer of fixed N_2 from peas to associated oats through the rapid mineralization of organic N derived from pea rhizodeposition may be another reason (Jensen, 1996). The higher photosynthetic rate of intercropped oats is most likely due to the higher N supply by the peas (Dordas and Sioulas, 2008), coupled with lower competitive ability of peas for light (Lima Filho, 2000). The response of oats in terms of photosynthesis rate and N uptake was stronger due to intercropping than due to organic fertilization.

In our study, oats were the dominant intercrop component in the control and compost treatments, while peas were the dominant intercrop component in the manure treatment. The peas were apparently less affected by interspecific competition in the manure treatment due to the lower oat density. This was also indicated by the higher nodule DW and N_2 fixation of the intercropped peas in manure plots compared to control and compost plots.

The positive residual effects of organic fertilizers on the succeeding winter wheat were distinctly more pronounced than in our experiment of the previous year (Jannoura et al., 2013), due to continuous decomposition of organic manures. Ghosh et al. (2004) showed that organic fertilizers applied to preceding crops leave a significant quantity of nutrients for the succeeding wheat. The highest winter wheat yield was recorded when wheat was preceded by sole pea compared with pea/oat intercrop or sole oat. This can be explained by (1) fixation of atmospheric N in pea and its residual effect on subsequent cereal crops (Hayat et al., 2008), (2) soil mineral N contents often being higher after grain legumes than after cereals (Hauggaard-Nielsen et al., 2009a), and (3) depletion of nutrients and immobilisation of nitrogen during the microbial decomposition of oat residues, which have slower rates of decomposition compared to that of pea (Karpenstein-Machan and Stuelpnagel, 2000; Ghosh et al., 2004).

6.4.2. Nodulation, N_2 fixation, and N uptake

Pea plants responded significantly to applied organic fertilizers in terms of nodule mass, N_2 fixation and photosynthesis, both in sole and intercrop systems. Increased legume nodulation due to organic manures has been repeatedly observed (Tagoe et al., 2008). This has sometimes been explained by improved P availability to the crops (Tagoe et al., 2008), an unlikely reason in our study since a similar amount of total P was added to all plots. Other reasons might be an improved soil environment in terms of

physical and chemical properties for nitrogenase activity (Ghosh et al., 2004) and the slow mineralization of N of organic fertilizers, improving nodulation (Otieno et al., 2009). The close relationship between average photosynthetic rate and nodule DW indicates the importance of photosynthesis for the supply of energy for nodule metabolism (Antolín et al., 2010). Peas amended with organic fertilizers were more vigorous (Supplementary Table 5) than those in the control treatments, indicating favourable growth conditions, contrasting the result of a similar experiment in the previous year (Jannoura et al., 2013), where organic fertilizers were less well mixed into the soil.

As in the previous experiment (Jannoura et al., 2013), manure and compost application did not enhance oat yields, although their application resulted in significantly higher N concentrations in grain and straw, suggesting the occurrence of N mineralization (Carefoot and Janzen, 1997). Compost provided 12 kg N ha^{-1} or 3.3% to sole cropped oats, but manure provided no additional N, calculating the N uptake as the difference between organic fertilizer and control treatments. Thus, the lack of response might be due to the very small amounts of organic N mineralised throughout the experiment. Temporary N immobilization in the straw-rich manure is also possible, especially in the first weeks after application (Bechini and Marino, 2009), when N demand of oats is high during this period of rapid growth. Cattle manure containing straw did not mineralise or immobilize measurable quantities of N in the first season after application (Thomsen and Kjellerup, 1997). An additional important factor responsible for the yield reductions in manure plots is the lower plant populations established in these plots. However, the growth and yield indices per plant were similar in all treatments (Supplementary Table 5).

6.4.3. Microbial root colonization and biomass indices

Root colonization by indigenous AMF was obviously stronger in peas than in oats, as observed by Jannoura et al. (2013). As in the pervious experiment (Jannoura et al., 2013), organic fertilizers significantly reduced mycorrhizal colonization of both crops, which has been reported for other species (Ellis et al., 1992; Tarkalson et al., 1998). This might be due to an increased microbial competition in the rhizosphere (Tarkalson et al., 1998) or the presence of available nutrients, reducing the dependency of plants on AMF (Ellis et al., 1992). Ergosterol concentrations in pea roots were roughly tenfold higher than in oat roots, suggesting that not only AMF, which do not contain ergosterol

(Olsson et al., 2003), but also saprotrophic fungi, which contain ergosterol (Djajakirana et al., 1996), colonized the N rich legume roots more strongly, as observed by Jannoura et al. (2013).

The addition of compost and especially manure significantly increased the ergosterol content and the ergosterol-to-microbial biomass C ratio, which suggests a shift in the microbial community structure towards saprotrophic fungi. Ergosterol is presently the most important indicator for fungal biomass in soil (Joergensen and Emmerling, 2006). This shift has been observed by Quintern et al. (2006a) after directly adding shredded shrubs and by Niklasch and Joergensen (2001) after adding yard-waste compost similar to the present compost. These organic fertilizers, which consist mainly of wooden debris, contain relatively large percentages of lignin and lignin-cellulose complexes, predominantly decomposed by fungi. In the case of horse manure, the increased fungal colonization may be due to the presence of wheat straw, which is strongly colonized by saprotrophic fungi and promotes these organisms after its application to soil (Scheller and Joergensen, 2008).

The application of organic fertilizers significantly increased soil microbial biomass C, N, and P at all sampling days. In most cases, this increase was significantly higher in the manure than in the compost treatments, despite the fact that equivalent amounts of organic C were added. This may be due to the higher contents of more readily decomposable C fractions in the horse manure (Rochette et al., 2006). It may also be due to the higher microbial biomass content in the manure itself (Gattinger et al., 2004). In this experiment, microbial biomass C, N and P in soil were positively correlated with DW yields of peas and with N concentrations in oat biomass but not with its DW yields. This indicates that horse manure has negative effects on growth conditions other than N uptake.

The present low ratios of microbial biomass C/N in combination with low microbial biomass C/P ratios indicate that microorganisms did not suffer from N or P deficiency (Joergensen and Emmerling, 2006). During crop maturation, microbial biomass C increased, while microbial biomass N remained nearly constant and microbial biomass P decreased, leading to significantly higher ratios of microbial biomass C/N and C/P ratios. This indicates that the C input derived from fresh crop residues and decaying roots exceeded the N and P input.

6.4.4. Decomposition of organic fertilizers

Soil CO_2 evolution rates in manure and compost treatments followed a temporal pattern similar to that in the control treatment, suggesting that mainly soil moisture and crop development controlled this pattern. Across all measuring dates, manure and compost application significantly increased CO_2 concentrations above the soil surface by 96 and 34% in comparison with the control treatment. Expressed as a percentage of organic C added (Eq. (5)), the average amounts of C mineralised as CO_2 in 133 days were 46% for manure but only 16% for compost. Thus, horse manure was mineralised more readily than compost, despite the fact that compost had a lower C/N ratio than manure (28 versus 39, respectively) and higher total organic N input (361 versus 258 kg N ha^{-1}, respectively). The higher mineralization rate of manure C may be due to the higher contents of more readily decomposable C fractions in the horse manure (Rochette et al., 2006). However, the proportion of CO_2-C mineralised from the present horse manure is in the lower part of the range reported for other animal manures under field conditions (Rochette et al., 2006). In the case of yard-waste compost, no data are available from field experiments in the literature for comparison.

The recoveries of POM–C and POM–N were decreased throughout the experiment, indicating substantial decomposition of the added substrate. At 101 DAS, according to the amounts of organic C recovered as total POM-C (0.4-2; >2 mm), significant amounts, namely 70 and 64% of organic C added with manure of compost, respectively, were lost during this period. However, not all organic material decomposed is necessarily mineralised as CO_2. Only 39 and 13% of C added with manure and compost, respectively, were evolved as CO_2-C during this period. The balance gaps of 28 and 50% for manure and compost may be caused by the formation of microbial residues (Muhammad et al., 2006). However, the strong C losses already obtained at the first sampling date suggest that an unknown percentage of the losses may be accounted for by unknown losses, e.g. the sampling procedure. An estimate of this bias might be obtained by sampling the soil directly after manure and compost application.

6.5. Conclusions

Application of C-rich organic fertilizers, such as yard-waste compost, but especially horse manure, greatly stimulated soil microbial biomass indices, which was reflected by increased pea yields in sole and intercropped systems. In contrast, compost and

especially manure application did not enhance oat yields, due to the poor seedling emergence. The shading effect of the intercropped cereal component has adverse effects on nodulation, N_2 fixation, photosynthetic rates and biomass of the intercropped legume component, but the LER values showed that intercropped plants used growth resources on average 10-20% more efficiently. According to the organic fertilizer recovered as POM as well as the CO_2 production, horse manure was more readily available to soil microorganisms than compost, leading to increased grain yields of the succeeding winter wheat. Organic fertilization and legume/cereal intercropping are important means for improving soil fertility, not only in organic farming systems, which demands more scientific and practical attention.

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7. Monitoring of crop biomass using true color aerial photographs taken from remote controlled hexacopter.

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Abstract

The use of an unmanned aerial vehicle has been recently increasing in precision agriculture as an alternative to very costly and not readily available satellites or airborne sensors. Vegetation indices based solely on visible reflectance, which can be derived from true color images may be a simple and cheap alternative compared to near infrared indices. A remote-controlled hexacopter with an RGB digital camera was tested for evaluating the crop biomass. The hexacopter was flown over a field in which peas and oats were grown as sole crops and intercrops, fertilised with horse manure and yard–waste compost (10 t C ha^{-1}). The images were taken at flowering stage. Based on the aerial photographs, the Normalised Green–Red Difference Index (NGRDI) was calculated, and related to aboveground biomass and leaf area index (LAI). The mean of NGRDI values ranged from 0.09 – 0.13 without any effect of cropping system, while

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the fertiliser significantly affected the yield and the corresponding NGRDI values. NGRDI values were positively and significantly correlated with the aboveground biomass (r = 0.58 – 0.78). A high autocorrelation of NGRDI, and thus biomass was found within the treatment plots and used for block kriging to show the spatial variability in the field. No relationship was found between NGRDI and LAI in peas (P = 0.68) and oats (P = 0.15). Nevertheless, true color images from a hexacopter and the derived NGRDI values are a cost-effective tool for biomass estimation and the establishment of yield variation maps for site-specific agricultural decision making.

Keywords: Hexacopter, True color images, aboveground biomass, NGRDI, Pea, Oat.

7.1. Introduction

Remote sensing has been successfully applied for monitoring crop growth and development during the growing season and for site specific management (Chang, Clay, Dalsted, Clay, & Neill, 2003; Swain, Thomson, & Jayasuriya, 2010). The traditional tools (e.g. satellites or conventional aircraft) are the primary platforms used to obtain remote sensing images. However, satellite and airborne sensors can be prohibitively expensive and inaccessible for researchers and farmers (Hunt, Cavigelli, Daughtry, McMurtrey, & Walthall, 2005; Robert, 2002). In addition, they have several critical disadvantages, such as a relatively low image resolution restricting their use to large scale applications and the limited availability of high quality imagery in time and space, which also depends on weather conditions and satellite sensor characteristics (Xiang & Tain, 2011).

Over the past decade, the development of light weight, unmanned aerial vehicles (UAVs) has offered a new solution for crop management and monitoring (Primicerio, Di Gennaro, Fiorillo, Genesio Lugato, Matese, & Vaccari, 2012). UAVs have several advantages: (1) they can be deployed quickly and repeatedly at low costs, (2) they are user friendly and flexible in terms of flying height and timing of missions and (3) they can deliver very fine image resolutions and are thus suitable for small-scale investigations (Colomina & Molina, 2014). The use of small rotary wing UAVs to remotely detect crop and soil properties may become a key factor for farmers in the future, with

promising capabilities for remote sensing applications in agriculture (Zhang & Kovacs, 2012; Primicerio et al., 2012).

Aerial photography with either true color or color infrared film is an appropriate technique for plant monitoring, providing quantitative information on the status quo and spatial variability for the whole study site. Vegetation indices obtained from aerial images can be used to estimate changes in the vegetation state, biomass, leaf area index and chlorophyll concentration (Swain et al., 2010; Gitelson, Viña, Arkebauer, Rundquist, Keydan & Leavitt, 2003; Gitelson, Kaufman, & Merzlyak, 1996). A widely used index for vegetation monitoring is the Normalised Difference Vegetation Index (NDVI), which is the ratio of the reflectance in the near-infrared and red portions of the electromagnetic spectrum (Tucker, 1979). Some other authors have used vegetation indices based solely on visible reflectance and using an RGB digital camera (Hunt et al., 2005; Rasmussen, Nielsen, Garcia-Ruiz, Christensen & Streibig, 2013; Torres-Sánchez, López-Granados, De Castro & Peña-Barragán, 2013).

The green-red ratio vegetation index (GRVI) or Normalised Green-Red Difference Index (NGRDI) are calculated from the reflectance in the green and red parts of the spectrum, which can be derived from true color images. These indices have been applied to monitor vegetation phenology (Motohka, Nasahara, Oguma, & Tsuchida, 2010), to determine aboveground biomass and nutrient status (Hunt et al., 2005) and for site-specific weed management (Torres-Sánchez et al., 2013). However, visual vegetation indices are not applied as often as near-infrared (NIR) indices, because the difference in digital numbers between the green and red bands for vegetation and soil is small compared with that between near-infrared and red bands (Hunt et al., 2005). NIR bands provide more information on the geometric features of crops and on biophysical parameters, such as the leaf area index (LAI) than visual bands (Breunig, Galvao, Formaggio & Epiphanio, 2013; Houborg & Boegh, 2008). Also, remote sensing technologies combined with spatial analysis make it possible to gain a detailed understanding of the spatial complexity of a field and its crop (Zhang, Lacey, Hoffmann, & Westbrook 2011). Spatial interpolation methods such as kriging can be used to create continuous surface maps. The resulting maps can be used as a model to provide spatially distributed information for site specific management (Basnyat, McConkey, Noble, & Meinert 2001; Zhang, Lan, Lacey, Hoffmann, & Westboork, 2011).

The field pea (*Pisum sativum* L.) is the most common grain legume in middle Europe for human nutrition and domestic protein fodder for farm animals. In organic farming, N_2 fixation of legumes, such as peas (*P. sativum* L.), is the main source of N input (Berry, Sylvester-Bradley, Phillips, Hatch, Cuttle, Rayns & Gosling, 2002). Oat (Avena sativa L.) is a cereal crop that is used throughout the world for human food and animal feed. The area harvested in Germany is 146,000 and 50,000 hectares of oats and peas, respectively (FAO, 2012). Legume-cereal intercropping is widely practiced, especially in organic farming systems, to enhance productivity, yield stability and land use efficiency of intercrops compared to sole crops (Jensen, 1996; Hauggaard-Nielsen & Jensen, 2001).

In the current study, a small remotely controlled hexacopter equipped with an RGB digital camera as an image sensor was tested for small-scale monitoring of crop biomass. The relationships between the NGRDI and aboveground plant biomass, and LAI were tested. In addition, NGRDI data obtained from true color aerial images of the field were tested for spatial autocorrelation to predict spatial distribution of aboveground biomass within a managed field.

The data analysis was based on a field experiment, which was established to examine the effects of organic fertiliser on the growth, crop yield, and soil microbial indices in sole and intercropped peas and oats under organic farming conditions (Jannoura, Joergensen, & Bruns, 2014) and monitored using ground measurements and high resolution true color aerial photographs.

7.2. Material and methods

7.2.1. Experimental design

The field experiment was carried out from April to August 2010 at the experimental farm of the University of Kassel, Frankenhausen in northern Hessia (51° 24' N, 9° 25' E, and 248 m above sea level), Germany (Jannoura et al., 2014). The 30 y mean annual rainfall is 698 mm. In 2010, the annual precipitation was 441 mm and thus 37% lower, with 97 mm falling in August. The long-term mean annual temperature is 8.5 °C. In 2010, the mean air temperature was 8 °C, the lowest and highest mean monthly values were –3.4 °C in January and 20.4 °C in July, respectively (Table 7.1). The air Temperature and precipitation were recorded by local meteorological station at Kassel-Calden airport (51° 25' N, 9° 23' E). The soil was classified as Haplic Luvisol (Quintern,

Joergensen & Wildhagen, 2006) and contained 17% clay, 81% silt, 2% sand, 1.3% total C and 0.15% total N. Soil pH (H_2O) was 7.0 and the water holding capacity was 49%.

Table 7.1: Temperature and precipitation for the year 2010 as compared to the long – term (1960-1990) at the study site.

Month	Temperature °C				Precipitation (mm)	
	2010			30 years	2010	30 years
	Minimun	Maximun	Average	Averange		
January	-10.8	1.8	-3.4	0.1	11	57
February	- 6.5	7.4	0.0	0.6	25	44
March	-6.2	13.4	4.6	4	35	54
April	4.9	18.5	9.1	7.9	7	53
May	5.4	17.2	10.3	12.4	51	66
June	10.7	21.2	15.9	15.6	40	80
July	15.1	27.3	20.4	17.1	23	63
August	9.7	21.4	16.6	16.9	97	62
September	8.4	16.2	12.5	13.7	54	56
October	2.5	15.9	8,4	9.5	19	46
November	-3.7	14.2	5.2	3.9	53	55
December	-9.6	3.9	-3.9	0,8	26	62

The crops were sown on 23 April 2010. Peas were sown at 80 seeds m^{-2} both in sole and intercropped plots, while oats were sown at 300 kernels m^{-2} in sole plots and at 60 kernels m^{-2} in the intercropped plots. Plant populations were determined by counting the number of seedlings in three randomly selected 1 m lengths of rows in each plot. The crops were cultivated according to organic agricultural practice and under rainfed conditions. Intercropped peas and oats were sown in the same row. The applied organic fertilisers were fresh horse manure, mixed with stall beddings (wheat straw) and rotted yard waste compost (shredded shrubs and tree clippings; 3 months old). Manure and compost were given to supply equivalent amounts of 10 t C ha^{-1} to the respective treatments.

Semi-leafless field peas (*P. sativum* L. var. Santana KWS, Einbeck) and oats (Avena sativa L. var Dominik) were grown as sole crops and as intercrops under three fertiliser treatments: (1) control without fertilisers, (2) horse manure and (3) yard-waste

compost. The trial was conducted in a split plot design with four replicates, using the fertiliser treatments as main plots and the three cropping treatments ((a) sole peas, (b) sole oats, and (c) peas and oats intercropped) as subplots (6 × 4.5 m) (Fig. 7.1; Fig.7.2). The subplots were separated from each other by a walking path of 0.5 m width. Each subplot was divided into three 6 × 1.5 m sub-subplots (Fig.7.1): one for final yield determination, where the plants were left undisturbed until harvest, and the other two for sampling and measurements. Sub-subplots consisted of seven rows with a row distance of 18.75 cm.

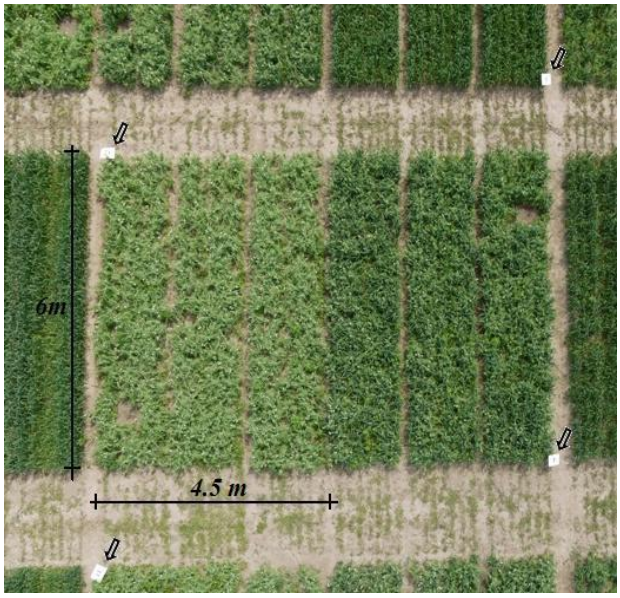

Fig.7.1: Aerial photograph of experimental plots at 60 days after sowing (DAS). Arrows indicate the ground control points, plot size (4.5 × 6 m).

7.2.2. Plant sampling

The crops were harvested on 24 June (early flowering of pea and late boot of oat) (62 days after sowing DAS). Aboveground plant biomass was determined by cutting the plants at soil level from an area of 1 m² of each plot. The material was separated into crop components (pea, oat and weeds), dried at 60 °C for 72 h and weighed. More details about the field experiment were described in Jannoura et al. (2014).

For determination of leaf area and LAI, three plants for each species per plot were selected randomly at 67 DAS, harvested and separated into leaves and stems. The leaf area of each plant was measured with a leaf area meter (Delta T device, UK) and total leaf area per plant was calculated by adding the area of individual leaves. LAI was calculated by multiplying the average leaf area per plant (m^2 $plant^{-1}$) by the total number of plants per m^2 (Jannoura et al., 2014).

(a)

b	OS+M	POI+C	PS	POI+C	OS+M	PS	d
	PS+M	OS+C	POI	PS+C	POI+M	OS	
	POI+M	PS+C	OS	OS+C	PS+M	POI	
a	POI	PS+M	OS+C	PS+M	OS	POI+C	c
	OS	POI+M	PS+C	POI+M	PS	OS+C	
	PS	OS+M	POI+C	OS+M	POI	PS+C	

Horse manure (M)
Compost (C)
Sole peas (PS)
Intercropped peas/oats (POI)
Sole oats (OS)
a, b, d, c Blocks

(b)

Fig.7.2: **(a)** Experimental design: main plots: three fertiliser treatments: no fertiliser (control), horse manure (M) and compost (C); sub-plots: three cropping systems: sole peas (PS), Intercropped peas/oats (POI) and sole oats (OS); each subplot was divided into three 6 × 1.5 m sub-subplots, n = 4. **(b)** The mosaic image for analysis in ArcGIS, Normalised Green–Red Difference Index (NGRDI) was estimated for $1m^2$ biomass sample area (the sign of the square).

7.2.3. Acquisition of aerial photographs and post-processing

Low altitude platforms such as a balloon (Buerkert, Mahler, & Marschner, 1996), model airplane (Hunt et al., 2005), and model helicopter (Swain et al., 2010) are increasingly being used as a tool to take aerial pictures in agricultural research. The development of new technologies is mainly due to the miniaturization of electronics and increasing weight reduction. The ability to take very high resolution images with drones increases their importance for small-scale investigations. Torres-Sánchez et al. (2013) used a quadrocopter and Rasmussen et al. (2013) used a hexacopter to gather high resolution images and subsequently to calculate a visible vegetation index. Their results indicated a great potential of these systems for site specific weed management (Rasmussen et al., 2013).

A commercially available remotely controlled hexacopter with six rotors (Flightcopter.TV, Germany) (Fig.7.3) equipped with a digital camera (Panasonic Lumix DMC-GF1) was used to obtain true color aerial images of the field. The weight of the hexacopter with camera was approx. 3 kg, resulting in a flight time of 10 min. The hexacopter was flown over the targeted area (1500 m²) at an altitude of approx. 30 m to capture many images with a resolution of 0.5 cm, whereby several plots were covered in one picture.

All aerial photographs were taken two days before plant harvest on 22 June (60 DAS) at noon during full sunshine to minimize shadow effects in photographs. For the georeferencing of the images during post-processing, 28 ground control points (GCPs) acquired by GPS (Leica SR 530 with horizontal accuracy of 1 – 2cm) were installed in the experimental plots (min 3 GCPs per plot) before the start of the flight mission (Fig. 7.1). After landing, images from the digital camera were downloaded to a laptop computer as JPEGs (Joint Photographic Experts Group files) and examined for completeness. Images were clipped to keep only the area of interest. These images were subsequently georeferenced with ArcGIS 9.2 (ESRI, Redlands, CA, USA) with reference to actual ground control points and converted into TIF format (Tagged Image File).

All images were combined to one image (mosaicking) depicting the whole experiental field using the Mosaic Tool of Erdas Imagine 9.2 (Leica Geosystems GIS & Mapping LLC., Norcross, GA, USA), whereby histogram matching was applied for color correction (Fig.2). Within ArcGIS 9.2 the Normalised Green–Red Difference Index (NGRDI) was calculated, using the formula of Hunt et al. (2005):

$$\mathrm{NGRDI} = \frac{(\text{Green DN} - \text{Red DN})}{(\text{Green DN} + \text{Red DN})}$$

where Green DN and Red DN are the digital numbers of the green and red bands, respectively. The close relationship between the NGRDI calculated from the camera and the NGRDI from the reflectances for the five colored tarpaulins indicated that the digital numbers can be used without further radiometric calibration (Hunt et al., 2005). Before the calculation, each band was transformed to a floating point pixel type (32 Bit) using ArcCatalog. NGRDI values can range from −1.0 to +1.0, where increasing positive values indicate increasing green vegetation and negative values indicate bare soil.

Fig.7. 3: Hexacopter used to take low-altitude aerial photographs.

7.2.4. Statistical and geostatistical analysis

Statistical analyses were carried out using SPSS statistical software (SPSS 15.0). The NGRDI values of the 1 m² biomass sample area were extracted and the mean value was calculated. The significance of experimental effects on plant biomass and NGRDI values was tested by a two-way ANOVA with fertiliser and cropping system as independent factors. The Spearman correlation coefficient was used to determine correlations between different variables. The independence of errors assumption was tested by residual analysis. Errors showed no correlation with independent variable ($R^2 = 3\times10^{-11}$), so independence of errors could be assumed.

To assess spatial variability of NGRDI, subplots of 1 m^2 per treatment and field replicate were defined. When possible, 9 subplots where placed in a 3×3 matrix within each 6×4.5 m plot, thus achieving a total of 309 points distributed systematically on the complete field. NGRDI were degraded from its original spatial resolution to 1 m to fit the 309 points. As the coordinates for each of the 309 points are known, a global semivariogram could be calculated for the complete trial using the 'gstat' package

(Pebesma, 2004) implemented in R (R Development Core Team, 2011). An exponential function was fitted to the data points to allow the calculation of range, sill and nugget parameters. An interpolated NGRDI map was generated by ordinary block kriging using the same package (Hengl, 2011) at a 1 m resolution. Based on the estimated NGRDI values, a biomass map was calculated using the correlation function described in Fig.7. 4d: Total aboveground biomass = 2164.3 NGRDI + 195.1

7.3. Results

The NGRDI values were not different for the three cropping systems (peas, oats, intercropped plots) but were significantly lower in manure treated plots (Table7.2). NGRDI values were positively and significantly correlated with total aboveground dry biomass (g m^{-2}) of sole pea plots (P = 0.02), intercropped plots (P = 0.08) and sole oat plots (P = 0.01) (Fig.7.4 a,b,c) and when combining the data over the three cropping systems (P = 0.001) (Fig.7.4d).

In this study, the relationship found between NGRDI and biomass, which were directly measured in the field (Fig.7.4d), was used to predict the biomass spatial distribution to create a final map of total biomass (Fig.7.5C). The exponential function in the NGRDI semivariogram (Fig. 5A) showed good autocorrelation up to 5.44 m distance (plot scale). Thus, the kriged map of NGRDI (Fig.7.5B) shows a high spatial autocorrelation within the treatment plots. For longer distances, the points are considered independent of each other and the semivariance no longer increases. The NGRDI values ranged between 0.04 and 0.16. Correspondingly, the estimated biomass ranged from 208 to 550 g m^{-2}.

Likewise, NGRDI values were positively and significantly correlated with pea biomass in sole pea plots (r = 0.59; $P < 0.05$), with oat biomass in sole oat plots (r = 0.78; $P < 0.01$), and with total pea and oat biomass in intercropped plots (r = 0.58; $P < 0.05$) (Fig.7.6), whereas the correlation coefficient of the combined data set, for all three cropping systems was low (r = 0.36; $P < 0.05$).

Table 7.2: Aboveground DM production and normalised green red difference index (NGRDI) under three cropping systems: sole peas, peas intercropped with oats and sole oats with three fertilizer treatments: control without fertilizer, horse manure and compost. Biomass was measured on 24 June (62 DAS), whereas NGRDI was measured two days before.

	Aboveground dry production (g m^{-2})				NGRDI
	Pea	Oat	Weed	Total	
Sole peas	239		135	374	0.13
Sole peas + manure	234		48	283	0.09
Sole peas + compost	235		146	381	0.10
Intercropped peas/oats	169	272	62	502	0.11
Intercropped peas/oats + manure	173	161	19	352	0.09
Intercropped peas/oats + compost	212	194	49	454	0.12
Sole oats		544	16	560	0.12
Sole oats + manure		357	9	365	0.09
Sole oats + compost		553	15	568	0.12
Probability values					
Cropping system	< 0.001	<0.001	<0.001	<0.001	NS
Fertilizer	NS	<0.001	<0.001	<0.001	<0.01
Crop x fertilizer	NS	NS	<0.001	NS	NS
CV (%)	11	15	33	11	22

CV = coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing, DM = dry matter.

At 62 DAS, horse manure application significantly decreased the total aboveground biomass in the three cropping systems. Organic fertiliser application had no effect on aboveground dry matter (DM) production of pea, while oat DM production was decreased significantly by manure application in intercropped and sole plots (Table 7.2). The weed DM production significantly decreased by manure application in all cropping systems. On average, weed DM production was only 13 kg ha^{-1} in sole oat plots and 3 and 8 times higher in intercropped and sole pea plots, respectively (Table7.2). The number of pea plants varied around 90 ±5 (standard error) in the sole plot and around 80±4 in the intercropping and that of the oat plants around 260±22 in sole plots and around 70±7 in the intercropping. The oats provided with manure had lower densities both in sole and intercropped systems.

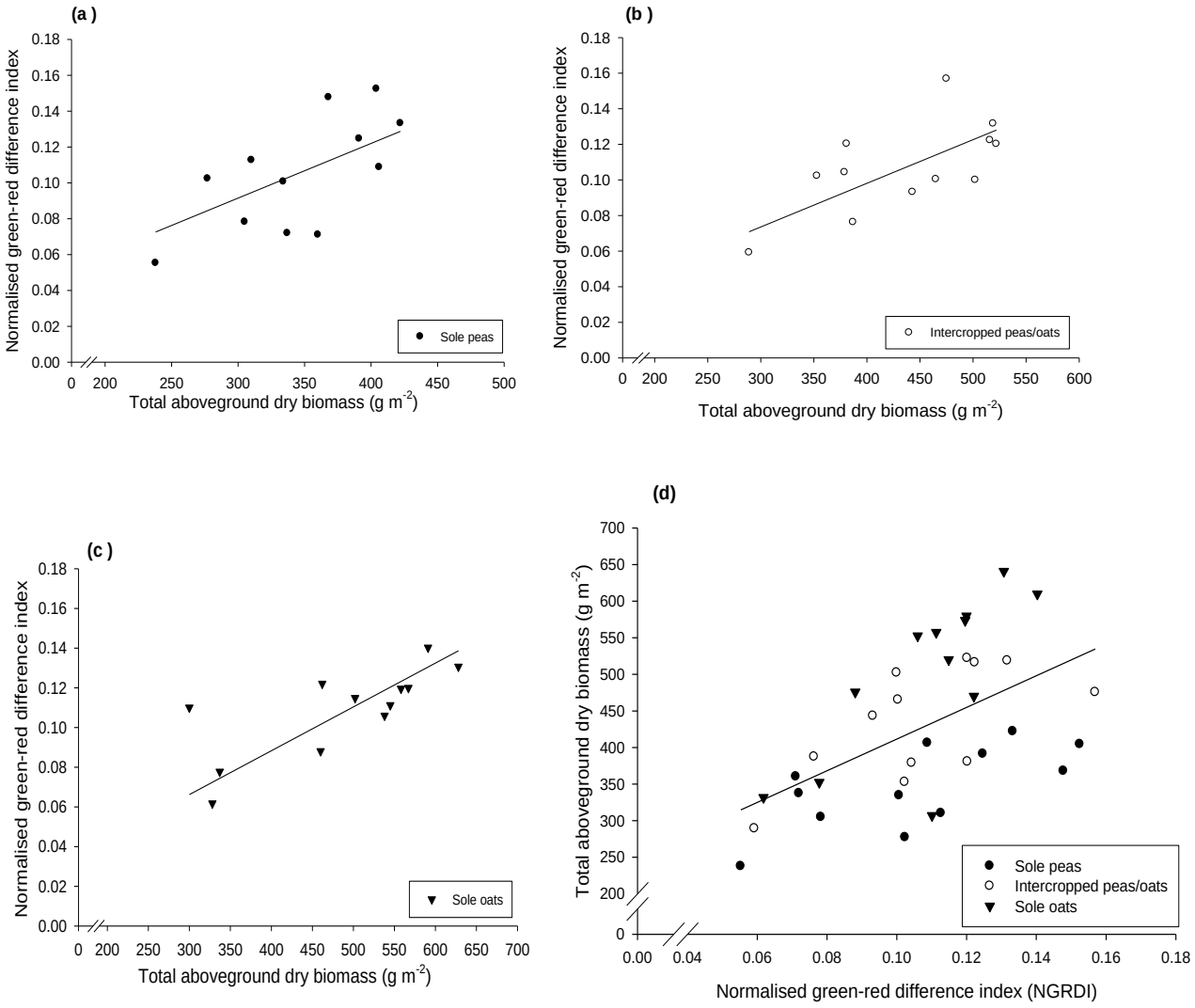

Fig.7.4: Relationship between normalised green-red difference index (NGRDI) and total aboveground dry biomass in three cropping systems: (a) sole peas (●; r = 0.65; P = 0.02; n = 12), (b) intercropped peas/oats (○; r = 0.55; P = 0.08; n = 12), (c) sole oats (▼; r = 0.74; P = 0.01; n = 12) and (d) when combining the data over the three cropping systems (Y= 2164.09X + 195.120; r = 0.55; P=0.001; n = 36), the solid line is a regression

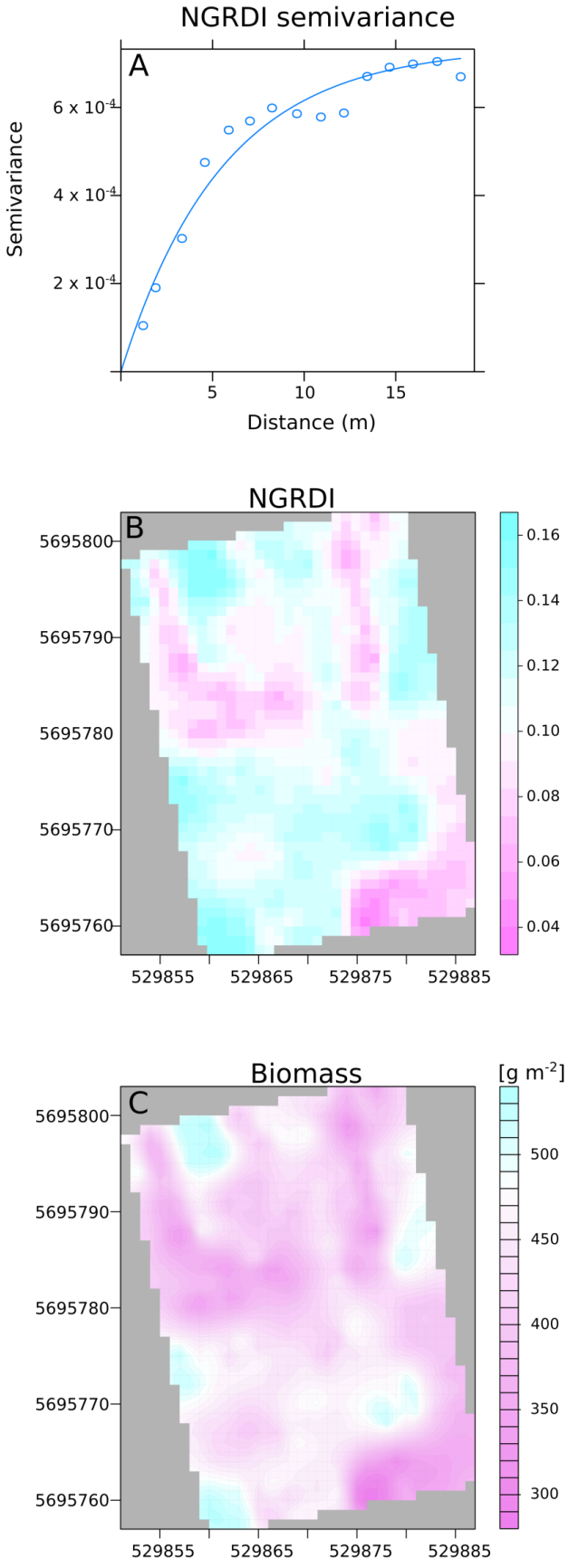

Fig.7.5: Semivariogram for NGRDI **(A)**; Kriged interpolation of normalised green-red difference index (NGRDI) (**B**); correlated spatial distribution of total aboveground dry biomass (g m^{-2}) (**C**).

The leaf area of pea and oat plants was on average 222 and 335 cm^2 $plant^{-1}$, respectively, without apparent effect of treatments (Table 7.3). The LAI of sole pea, and oat were on average 2.3 and 8.7, respectively. Under intercropping conditions, these values dropped to 1.7 and 2.2. The combined LAI in intercropped plots was on average 3.8. The application of organic fertiliser significantly decreased leaf area and LAI in sole and intercropped oat. No relationship was observed between the vegetation index and LAI (Fig.7.7). The leaf area and LAI of sole pea were also mentioned as supplementary data in Jannoura et al. (2014).

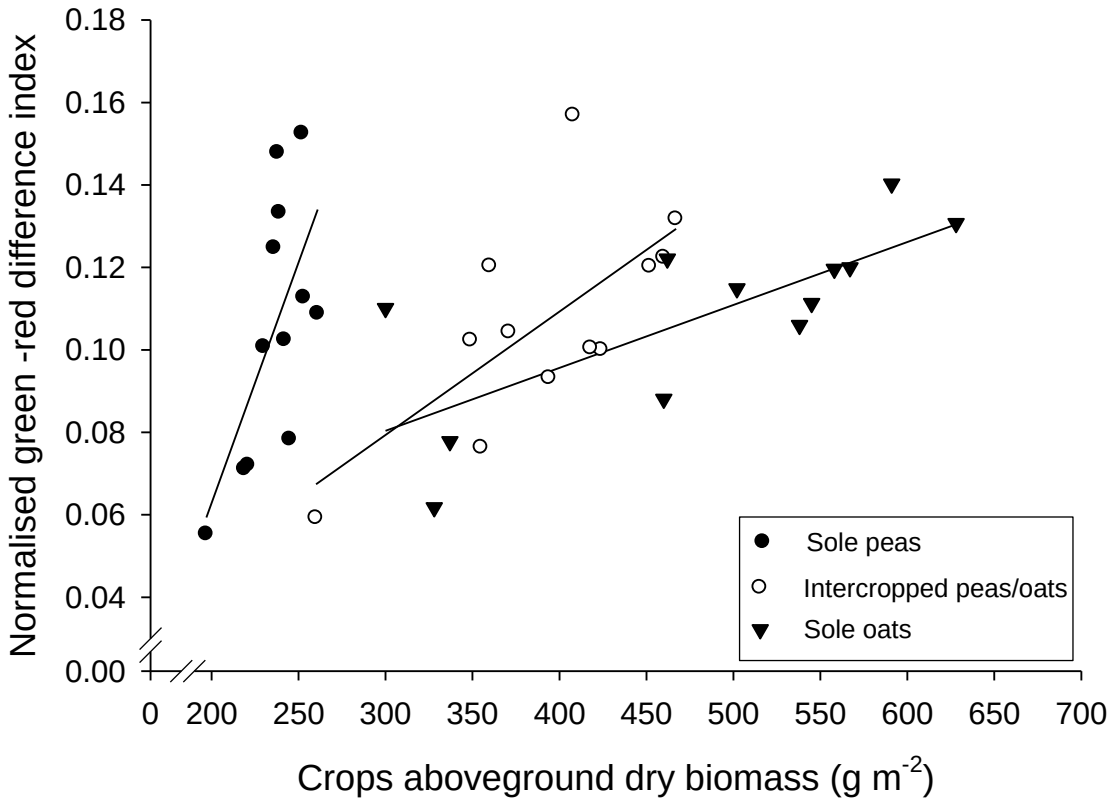

Fig.7.6: Relationship between normalised green-red difference index (NGRDI) and crop aboveground dry biomass in three cropping systems: sole peas ($r = 0.59$; $P < 0.05$), intercropped peas/oats ($r= 0.58$; $P < 0.05$) and sole oats ($r = 0.78$; $P < 0.01$).

7.4. Discussion

7.4.1. Aerial Photography

The hexacopter used in this study provides an interesting and relatively simple technique for obtaining aerial photographs of the area of interest. This system is an easily transportable platform that is able to fly precisely over a fixed point. During the

flights, a series of overlapping photographs were successfully taken to cover the entire area of the field experiment. The images obtained were of good quality, and the high resolution image mosaic of the entire field experiment was subsequently used for calculating the vegetation index.

However, the pixel intensity is affected by changes in solar irradiation, so the same ground location may have different numbers in sequential images. Additionally, uncalibrated cameras often have illumination variations, shadows and other elements that can hinder analysis by image processing techniques. The camera itself in this experiment was not calibrated, we previously thought that the radiometric calibration of camera is especially important for comparing different places and times (Hunt, Hively, Fujikawa, Linden, Daughtry, & McCarty, 2010), but not for investigating a site as small as the current experimental plot. Hunt et al. (2005, 2010) used five colored tarpaulins (beige, gray, green, red, and black) to calibrate the digital photographs, while Swain et al. (2010) used a spectroradiometer with wavelength range of 350 to 2350 nm to estimate reflectance at ground level in the red and NIR bands and compared it with image derived measurements based on NDVI calculations. In our study, we performed only a color balancing between images with histogram matching during the process of image mosaicking (Fig.7.2) to reduce variations of the input images. The histogram matching of images in this experiment has functioned well, but it would be much better, if the camera was calibrated, which should be made in the next experiments. In addition, it is recommended that the hexacopter be provided with an altimeter for monitoring flight altitude in order to choose the images closest to the same height (Gérard, Buerkert, Hiernaux, & Marschner, 1997; Swain et al., 2010).

7.4.2. NGRDI, biomass, LAI

A significant relationship was observed between NGRDI and aboveground biomass. However, these correlations were not as strong as those reported by Hunt et al. (2005), who observed high correlations ($r = 0.63 – 0.94$) between NGRDI and dry biomass for soybeans, alfalfa and corn when the yield was lower than 120 g m^{-2}. NGRDI was saturated for biomass greater than 150 g m^{-2} in soybean and corn, which indicated that NGRDI may be sensitive to biomass before canopy closure (Hunt et al., 2005).

At this stage of plant growth, the mean NGRDI values varied only between 0.09 and 0.13 and were within the range reported by Hunt et al. (2005) for other crop species. These values were small compared to those of other vegetation indices derived from red

and near-infrared reflectance (NDVI) (Gutierrez-Rodriguez, Escalante-Estrada, & Rodrigues-Gonzales, 2005) or from green and near-infrared (GNDVI) (Gitelson et al., 1996). In this study using the visible spectrum, averaged across fertiliser treatments, NGRDI was 0.11 in the three cropping systems, without any effect of crop species or cropping system. In contrast to this, Sharaiha and Ziadat (2008) reported higher NDVI values (0.24 – 0.26) in plots cultivated with sole barley compared to plots cultivated with sole vetch (0.13 – 0.16).

Table 7.3**:** Leaf area and leaf area index (LAI) of pea and oat at 67 DAS under three cropping systems: sole peas, peas intercropped with oats and sole oats with three fertilizer treatments: control without fertilizer, horse manure and compost.

Treatment	Leaf area (cm^2 $plant^{-1}$)		LAI		
	Pea	Oat	Pea	Oat	Total
Sole peas	223		2.2		2.2
Sole peas +manure	270		2.6		2.6
Sole peas + compost	213		2.1		2.1
Intercropped peas/oats	187	360	1.5	2.5	4.0
Intercropped peas/oats + manure	223	286	1.8	2.0	3.8
Intercropped peas/oats + compost	214	275	1.7	2.0	3.7
Sole oats		375		9.4	9.4
Sole oats+ manure		326		8.2	8.2
Sole oats + compost		345		8.6	8.6
Probability values					
Cropping system	0.04	0.07	<0.01	<0.01	<0.01
Fertilizer	0.03	0.05	0.08	0.08	NS
Crop x fertilizer	NS	NS	NS	NS	NS
CV (%)	24	17	17	36	16

CV = coefficient of variation between replicate plots (n = 12), NS = not significant.

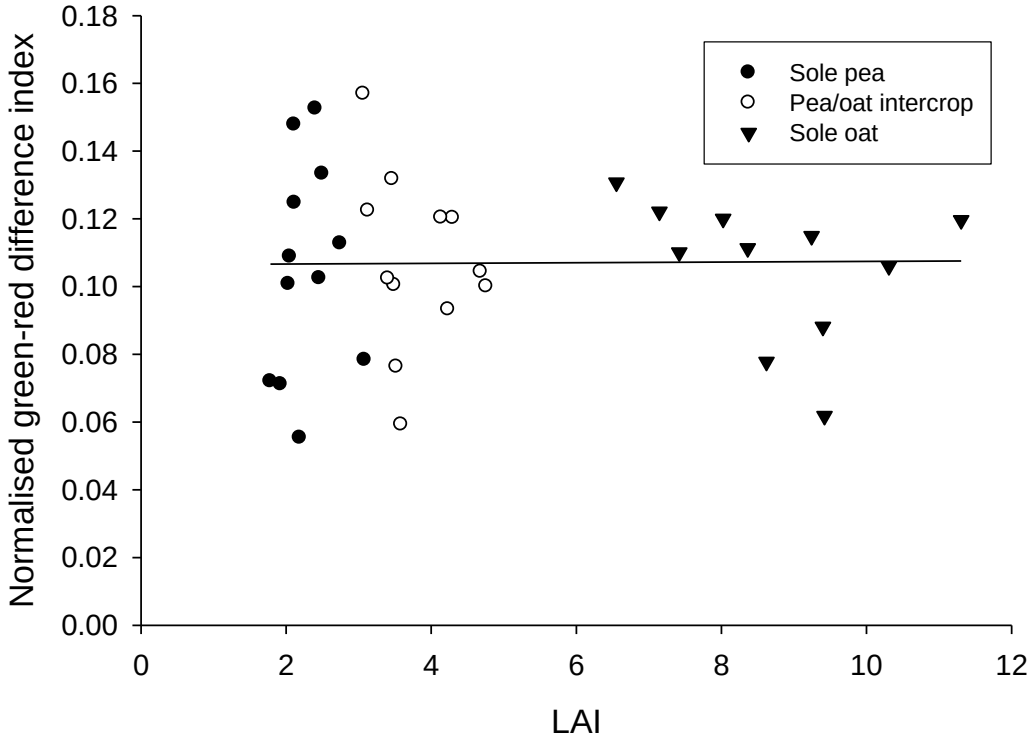

Fig.7.7: Relationship between normalised green-red difference index (NGRDI) and LAI in three cropping systems: sole peas, intercropped peas/oats and sole oats. Biomass was measured on 24 June (62 DAS), whereas LAI was measured at 67 DAS.

At this stage of crop development, LAI ranged from 2.1 in sole pea plots to more than 9 in sole oat plots, while a close relationship was found between aboveground biomass and LAI ($r = 0.78$, $P < 0.01$). Although no correlation could be detected between LAI and NGRDI, Hunt et al. (2005) found that NGRDI approaches a saturation level at about 0.05 when LAI of corn grown under three nitrogen levels exceeds 2. However, some reflectance indices (NDVI, NGRDI) are generally insensitive to LAI variations, as LAI reaches high values of approximately 2 to 3 (Carlson & Ripley, 1997; Hunt et al., 2005). The saturation level depends on the type of vegetation index used, the crop studied, and also the experimental conditions (Baret and Guyot 1991). Many other factors can affect the correlation between vegetation indices and LAI, such as soil background, canopy senescence and sun position (Gamon, Field, Goulden, Griffin, Hartley, Joel, Penuelas, & Valentini, 1995). However, the geometric structure of the plant (e.g. the leaf orientation, i.e., leaf angles; leaf dispersion; spatial distribution of the leaf area), plant architecture and many internal and external factors affect the canopy reflectance, which make the relationships between the LAI and the VI difficult (Goal & Qin, 1994).

The vegetation indices, which are more dependent on NIR band, are much more sensitive to LAI (Breunig et al., 2013).

7.4.3. Geostatistical analysis

The remote sensing data or aerial photographs analyzed by a geostatistical method, variogram, and kriging, provide a good description of the spatial variation within the field (Zhang et al., 2011; Dungan, 1998). Indices relative to the biomass (such as greenness index and NDVI) are often used for evaluating biomass amount and vegetation health. NDVI have been frequently used to create maps of biomass in forest management (Santi, Tarantino, Amici, Bacaro, Blonda, Borselli, Rossi, Tozzi, &Torri, 2014; Kumar, Sharma, Pandey, Sinha, & Nathawat, 2013), but also in large fields to predict and map variation of wheat grain yield (Basnyat et al., 2001) or soybean (Basso, Ritchie, Pierce, Braga, & Jones, 2001). In this study, the final map of biomass distribution in the field was created using the correlation between the NGRDI index and the measured biomass.

In the present study field of 0.15 ha, the spatial autocorrelation of NGRDI was 5.44 m (fitted range parameter). This is much lower than common field results, e.g. Zhang et al. (2011) who found a spatial dependence of the NDVI of 40 m with a sampling grid area of 4×3 m in soybean fields. This great difference is explained because of the sampling scale used (1 m^2 for NGRDI and biomass) and the plot size of the different treatments (6.0×4.5 m). The spatial correlation of NGRDI in this field showed autocorrelation within the plots for each treatment which was expectable due to the plot distribution. This means that at distances greater than 5.44 m the biomass spatial variability could only be explained by the treatments and other external influences (e.g. the different weed distribution among the field). Basso et al. (2001) showed that remote sensing imageries used in conjunction with the NDVI made it possible to identify spatial patterns of crop growth variability. A good correlation between the measured and estimated aboveground biomass ($n = 36$) was observed ($r = 0.60$; $P = 0.0001$), which indicates that the NGRDI is a good estimator for the aboveground biomass. In Basnyat et al. (2001) a correlation between wheat grain yield and the NDVI was also observed ($r = 0.62$). True color images with NGRDI analysis made it possible to identify spatial patterns of biomass variability. It would be worthwhile to study different sampling areas, growth stages and vegetation indices in a future study.

7.5. Conclusion

Our first experiment shows that the small low-altitude hexacopter is a rapid, simple and cheap technique for acquiring high resolution images to be used in precision agriculture. The observed correlation between NGRDI and aboveground biomass indicates that true color images allow to determine crop biomass and to establish yield variation maps on an entire field. The fit between the spatial distribution of the NGRDI and the total aboveground biomass has been shown to be a critical factor in precision agriculture. The calculated VI, not including NIR information, failed to detect differences between cropping systems and was not correlated with LAI. The current results are encouraging for the development of UAVs as a tool for site-specific precision agriculture in a small field area.

Acknowledgements

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8. Summary

Organic fertilizer application and microbial decomposition processes play essential roles in organic farming systems to maintain soil fertility and increase crop productivity. In addition, biofertilizers such as AMF are an important component of integrated nutrient management. AMF play a central role in ecosystem functioning by influencing nutrient fluxes and their interaction with other microorganisms in the rhizosphere. Moreover, there is some experimental evidence for the role of AMF in decomposition processes in soil, although AMF are obligate biotrophic organisms. Legumes such as pea (*Pisum sativum* L.) remain the main source of N input in organic farming. However, field peas grown as a sole crop suffer many problems, such as yield variability, lodging and weak competitive ability towards weeds. The strategies that aim to increase the yield of legumes have an important place in organic farming.

This thesis represents interdisciplinary research at the interface between soil biology, decomposition processes, and crop science, and is based on five experiments: two pot experiments (Chapter 3 and 4), two field experiments (Chapter 5 and 6), and an evaluation of the crop biomass using aerial photographs of the second field experiment (Chapter 7). In the two pot experiments, two pea isolines, the non-mycorrhizal mutant P2 (myc^-) and the symbiotic isoline Frisson (myc^+), were examined in the presence or absence of ^{15}N-labeled maize residues. The first pot experiment was carried out to study the effects of pea growth and AMF on the decomposition of ^{15}N-labeled maize residues, to compare the yield between the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson and to follow the distribution of the added substrate over different soil and plant fractions, which make it possible to distinguish between the use of added C4 material and that of soil organic matter derived from C3. However, in the first greenhouse experiment, using ink staining, no mycorrhizal colonization was observed in roots of the *myc*+ plants, which probably indicates that the fertilizer rates used were too high. Therefore, this experiment was listed as a preliminary experiment in this work and not all analyses in soil and plants were performed. This experiment was repeated again using lower fertilizer rates.

In the first greenhouse experiment (Chapter 3), the myc^- plants showed no growth differences compared to the myc^+ plants with the exception of roots. The amount of root C of the myc^+ plants was higher by 65%. The addition of maize leaf straw (C/N ratio of 17) significantly increased the contents of microbial biomass C, N and P at day 0 and 7. At the end of the experiment, the effect of growing pea roots alone on the microbial

biomass C was negligible. In contrast, the combination of growing pea plants and maize straw significantly increased the content microbial biomass C. On the basis of the difference between straw-C added and straw-C recovered, 92% of the straw added was decomposed in unplanted soil and 87% in the presence of plants, indicating nearly complete decomposition during the 101 day experimental period. The presence of living roots reduced the decomposition rate of the added maize straw.

In the second pot experiment (Chapter 4), lower fertilizer rates were applied to provide adequate nutrients for the growth of the *myc*$^-$ peas, while maintaining good mycorrhizal colonization of *myc*$^+$ roots. Mycorrhizal infection was well established in the roots of the *myc*$^+$ plants at flowering, but the level of colonization was strongly decreased at maturity. Yields of C in straw, grain and roots of *myc*$^+$ peas were significantly higher by 27, 11, and 92%, respectively, compared with those of *myc*$^-$ peas. The proportion of plant N derived from fixation of the *myc*$^+$ Frisson was 29% in the *maize*$^+$ treatment but only 5% in the *maize*$^-$ treatment. The presence of maize residues alleviates the negative effect of N fertilizer on N_2 fixation, probably due to microbial immobilization of available inorganic N. An interesting, unknown feature is the observation that the $\delta^{13}C$ values in the different plant parts were significantly higher in *myc*$^+$ than in *myc*$^-$ tissue with and without maize. This can be explained by the higher depletion of $\delta^{13}C$ by dark respiration or by a higher water use efficiency of the pea plants in the presence of AMF, leading to higher $\delta^{13}C$ values in plant tissues. The $\delta^{15}N$ values in the *maize*$^+$ treatment tended to be significantly lower in the *myc*$^+$ plants, indicating a lower uptake of maize-derived N caused by the lower decomposition rate of maize residues in this treatment, but also may be the contribution of the unlabeled N from fixation of atmospheric N_2. The ergosterol concentration in roots of mature peas did not differ between the two isolines, indicating similar colonization by saprotrophic fungi. The decomposition rate of added maize residues was significantly reduced by the presence of peas, especially by *myc*$^+$ peas, indicating that AMF are not involved in the decomposition of newly added maize residues. The recovery of maize residue C and N in the two POM fractions was roughly 6% in the *pea*$^-$ soil and increased to 14% and 18% in the presence of *myc*$^-$ and *myc*$^+$ roots, respectively. The substrate use efficiency was higher in the presence of pea plants, especially *myc*$^-$ peas. The formation of microbial residue C was increased and that of microbial residue N was reduced in the presence of plants. The insufficient N supply to soil microorganisms reduced decomposition of maize residues in the presence of peas, especially *myc*$^+$ peas.

The third and the fourth experiments were field experiments. They were conducted under organic farming conditions using C-rich organic fertilizers. The effects of different quality and quantity of C were considered. The organic fertilizers used were horse manure mixed with stall bedding, and yard-waste compost derived from shrub and garden cuttings. In these two experiments, sole and intercrops of field peas (*Pisum sativum* L.) and oat (*Avena sativa* L.) were investigated.

In the third experiment (Chapter 5), horse manure and compost were supplied at nearly equivalent N amounts but different C amounts to peas (*Pisum sativum* L.), either as a sole crop or intercropped with oat (*Avena sativa* L.). The objectives were: (1) to evaluate the beneficial effects of manure and compost on pea productivity, (2) to investigate whether these effects were reflected by microbial root colonization, microbial biomass and CO_2 production, and (3) to study the residual effects of the organic fertilizers on the yield of succeeding crop. Short-term application of horse manure and compost greatly stimulated soil microbial biomass C, N, P, fungal ergosterol, and CO_2 evolution, but failed to stimulate productivity of the current crops. The lack of response cannot be solely attributed to N and P deficiency, as both crops grown in the organic fertilizer treatments exhibited significantly higher P and N concentrations in grain and straw than those grown in the control treatments, but also to S deficiency, and others factors that caused poor germination, emergence, and early plant growth. However, significant positive residual effects of organic fertilizers, especially horse manure, were observed on the grain yield of the succeeding winter wheat, suggesting that the slow release of nutrients from the decomposition of organic fertilizers has significant positive long-term effects. Mycorrhizal colonization and ergosterol concentration were significantly higher in pea than in oat roots. Intercropping is an important tool for controlling weeds in pea plots under organic farming conditions, but did not affect microbial root colonization, soil microbial biomass indices or CO_2 evolution from the soil surface. According to the extrapolation of the CO_2 evolution rates into amounts per hectare, approximately 40% of the manure C and 24% of the compost C were mineralized to CO_2 during the 124-day experimental period. A noteworthy result of this study is the positive correlations observed between microbial biomass indices C, N and P in the soil and the concentrations of N and P in plant material of both species, suggesting that soil microbial biomass can be used as an indicator of nutrient availability to plants.

In the fourth experiment (Chapter 6), peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) were grown as sole crops and intercrops, fertilized with horse manure and yard-waste compost at 10 t C ha^{-1} each. The objectives were to compare the effects of these organic fertilizer and cropping systems in organic farming on (a) yield of peas and oats, grown as the sole crop or intercropped, as well as N_2 fixation and photosynthetic rates, (b) the yield of wheat as a succeeding crop, (c) microbial biomass indices in soil and roots, and (d) microbial activity estimated by the CO_2 evolution rate in the field and the amount of organic fertilizers, recovered as particulate organic matter (POM). In this experiment, positive effects of organic fertilizers on pea yield were observed. In general, organic fertilizer application improved nodule dry weight, photosynthetic rates, N_2 fixation, and N accumulation of peas as well as N concentration in oat grain. Averaged across fertilizer treatments, pea/oat intercropping significantly decreased nodule dry weight, N_2 fixation and photosynthetic rate of peas by 14, 17, and 12%, respectively, due to shading of peas by the oat canopy, and significantly increased the photosynthetic rate of oats by 20% due to the intraspecific competition in sole oats. However, the land equivalent ratio (LER) of intercropped peas and oats exceeded 1.0, indicating a yield advantage over sole cropping. Highly significant relationships were observed between pea yields and N uptake, nodulation, N_2 fixation and mean photosynthetic rate ($r = 0.71 - 0.77$), indicating the contribution of these indices to the higher crop productivity. Soil microbial biomass was positively correlated with pea dry matter yields both in sole and intercropped systems. Organic fertilizers increased CO_2 production and the contents of microbial biomass C, N, P, and fungal ergosterol in soil, whereas cropping system had no effects on these microbial indices. According to the organic fertilizer recovered as POM, 70% (manure) and 64% (compost) of added C were decomposed, but only 39% (manure) and 13% (compost) could be attributed to CO_2-C during a 101-day period. This indicates that horse manure was more readily available to soil microorganisms than compost, leading to increased grain yields of the succeeding winter wheat.

In the fifth experiment (Chapter 7), high-resolution true color photographs were taken by a small remote-controlled hexacopter, and ground measurements were conducted to monitor the treatment effects of the second field experiment. The images were taken at flowering stage, 60 days after sowing. Based on the aerial photographs, the Normalised Green–Red Difference Index (NGRDI) was calculated, and related to aboveground biomass and leaf area index (LAI). The mean of NGRDI values ranged

from 0.09 – 0.13 without any effect of cropping system, while the fertilizer significantly affected the yield and the corresponding NGRDI values. NGRDI values were positively and significantly correlated with the aboveground biomass (r = 0.58 – 0.78). A high autocorrelation of NGRDI, and thus above-ground biomass, was found within the treatment plots and was used for block kriging to show the spatial variability in the field. No relationship was found between NGRDI and LAI in peas (P = 0.68) and oats (P = 0.15). Nevertheless, true color images from a hexacopter and the derived NGRDI values are a cost-effective tool for biomass estimation and the establishment of yield variation maps for site-specific agricultural decision making.

9. Zusammenfassung

In der ökologischen Landwirtschaft spielen organische Düngemittel und deren mikrobielle Zersetzung eine essentielle Rolle, um die Bodenfruchtbarkeit zu erhalten und die Erträge zu steigern. Zusätzlich sind Biodünger wie arbuskulären Mykorrhizapilze (AMP) ein wichtiger Bestandteil des integrierten Nährstoffmanagements. AMF kann Nährstoffflüsse im Boden beeinflussen und interagiert auf vielfältige Weise mit den Mikroorganismen der Rhizosphere. Somit spielt das Vorhandensein von AMF eine zentrale Rolle in Ökosystemen. Obwohl AMP obligat biotrophe Organismen sind, gibt es zudem experimentelle Hinweise auf deren Beteiligung an Abbauprozessen im Boden.

In der ökologischen Landwirtschaft stellen Leguminosen wie Erbsen (*Pisum sativum* L.) die Hauptquelle für den Stickstoffeintrag in den Boden dar. Jedoch können bei Felderbsen, die in Reinsaat angebaut werden, viele Probleme entstehen wie z.B. Ertragsschwankungen, schlechte Standfestigkeit oder eine schwache Konkurrenzfähigkeit gegenüber Unkräutern. Strategien zur Ertragssteigerung von Leguminosen sind daher in der ökologischen Landwirtschaft von großer Bedeutung.

Die vorliegende Arbeit basiert auf fünf Experimenten, bestehend aus zwei Gefäßversuchen (Kapitel 3 und 4), zwei Feldversuchen (Kapitel 5 und 6) und aus Luftbildern des zweiten Feldversuches zur Abschätzung der Pflanzenbiomasse (Kapitel 7). In beiden Gefäßversuchen wurden zwei Erbsenlinien, die nicht mykorrhizierende Mutante P2 (myc^-) und der symbiotische Wildtyp Frisson (myc^+) in Kombination mit ^{15}N-markierten Maisrückständen untersucht. Die Gefäßversuche wurden durchgeführt, um 1.) die Auswirkungen des Erbsenwachstums und der AMF auf den Abbau ^{15}N-markierter Maisrückstände zu untersuchen, 2.) den Ertrag zwischen der nicht

mykorrhizierende Mutante P2 und des symbiotischen Wildtyps Frisson zu vergleichen und 3.) die Nährstoffflüsse des zugegebenen Substrates in verschiedene Kompartimente des Bodens und der Pflanzen zu verfolgen und damit eine Differenzierung der Substratnutzung zwischen dem zugegebenen pflanzlichen Material (C_4) und der organischen Bodenmaterial (C_3) zu ermöglichen. Der erste Gewächshausversuch zeigte keine Mykorrhizierung der *myc*$^+$-Pflanzen, was auf eine starke Nutzung der eingesetzten Dünger schließen lässt. Daher wird dieses Experiment in der vorliegenden Arbeit als Vorversuch beschrieben, in welchem nicht alle Analysen der Pflanzen und des Bodens durchgeführt wurden. Dieser Versuch wurde anschließend mit geringeren Düngergaben wiederholt.

Im ersten Gewächshausversuch wurde mit Ausnahme der Wurzeln kein Unterschied im Wachstum der *myc*$^-$ und *myc*$^+$-Pflanzen festgestellt. Jedoch war der Kohlenstoffgehalt der Wurzeln der *myc*$^+$-Pflanzen um 65% erhöht. Durch die Zugabe von Maisblattstreu (C/N von 17) wurde sowohl mikrobielles C, als auch mikrobielles N und P an Tag 0 und Tag 7 signifikant erhöht. Zu Versuchsende konnte festgestellt werden, dass Erbsenwurzeln keinen Einfluss auf das C der mikrobiellen Biomasse haben, wenn auf die Zugabe von Maisstreu verzichtet wird. Die Kombination aus Erbsenpflanzen und Maisstreuzugabe führte dagegen zu signifikant höheren Mengen an C in mikrobieller Biomasse. Die Differenz zwischen zugegebenem und wiedergefundenem Maisstreukohlenstoff zeigte, dass ohne Bepflanzung 93% und in Anwesenheit der Erbsen 87% des zugegebenen Kohlenstoffes abgebaut wurden. Dies deutet auf einen fast kompletten Abbau während des 101tägigen Experiments hin. Lebende Wurzeln verringerten die Abbaurate der applizierten Maisstreu.

Im zweiten Gewächshausversuch wurde Dünger in niedrigeren Mengen appliziert, um eine gute Mykorrhizierung der *myc*$^+$ Pflanzen zu gewährleisten. Bis zur Blüte konnte eine hohe Mykorrhizierung der *myc*$^+$ Wurzeln erreicht werden. Diese nahm jedoch bis zur Reife wieder stark ab. In den *myc*$^+$ Erbsen war der C-Gehalt im Stroh, in den Körnern und in den Wurzeln um 27%, 11% bzw. 92% gegenüber den *myc*$^-$ Erbsen erhöht. Der aus der N_2-Fixierung stammende Anteil des pflanzlichen N von *myc*$^+$ Frisson betrug in der *maize*$^+$ Behandlung 29%, in der *maize*$^-$ Behandlung jedoch nur 5%. Das Einbringen von Maisrückständen führte vermutlich zur mikrobiellen Immobilisierung des verfügbaren anorganischen Stickstoffes. Somit wurden die möglichen negativen Auswirkungen der N-Düngung auf die N_2-Fixierung der Pflanzen gemildert. Im *myc*$^+$ Gewebe der Erbse konnten stets höhere $\delta^{13}C$-Werte als in *myc*$^-$

Gewebe gefunden werden, unabhängig vom Vorhandensein von Maisrückständen. Dies kann einerseits durch eine $\delta^{13}C$ Verarmung während der Nachtatmung, andererseits durch eine erhöhte Wassernutzungseffizienz der Erbsen in Anwesenheit von AMF erklärt werden, was zu höheren $\delta^{13}C$-Werten im Pflanzengewebe führt. Die $\delta^{15}N$-Werte der *myc*$^+$ Pflanzen der *maize*$^+$ Behandlung waren signifikant niedriger, was auf eine geringer Aufnahme des maisbürtigen N hindeutet. Dies kann durch die niedrigere Abbaurate der Maisrückstände in dieser Behandlung hervorgerufen worden sein, aber auch auf eine Beteiligung des nicht markierten N aus der atmosphärischen N_2-Fixierung hindeuten. Der Ergosterolgehalt in den Wurzeln der reifen Erbsen unterschied sich nicht zwischen beiden Erbsenlinien, was auf eine ähnliche Besiedelung durch saprotrophe Pilze hinweist. Der Abbau der zugefügten Maisrückstände wurde durch die Erbsen signifikant verringert, besonders in der *myc*$^+$ Variante. Dies deutet darauf hin, dass AMF nicht am Abbau der zugegebenen Maisrückstände beteiligt sind. Ungefähr 6% des maisbürtigen C und N wurden in beiden POM Fraktionen des *pea*$^-$ Bodens wiedergefunden. Die Wiederfindung stieg in Anwesenheit von *myc*$^-$ und *myc*$^+$ Wurzeln auf 14 bzw. 18% an. In Anwesenheit der Erbsen, besonders der *myc*$^-$ Variante, wurde eine höhere Substratnutzungseffizienz festgestellt. In Anwesenheit der Pflanzen war die Bildung von mikrobiellem Residual-C erhöht, das N in den mikrobiellen Residuen war hingegen geringer. Die unzureichende N-Versorgung der Mikroorganismen verringerte den Abbau der Maisrückstände in Anwesenheit der Erbsenpflanzen, besonders in der *myc*$^+$ Variante.

Die Versuche 3 und 4 wurden unter Freilandbedingungen in den Jahren 2009 bzw. 2010 durchgeführt. Beide Versuche wurden mittels C-reicher organischer Dünger unter Anbaubedingungen des ökologischen Landbaus durchgeführt. Dabei wurden die Auswirkungen unterschiedlicher C-Mengen und C-Qualitäten untersucht. Als organische Dünger wurden strohreicher Pferdemist und Grüngutkompost, bestehend aus Strauch- und Gartenschnittresten, verwendet. In beiden Experimenten wurden sowohl Reinsaat, als auch Mischsaaten von Erbsen (*Pisum sativum* L.) und Hafer (*Avena sativa* L.) untersucht.

Im dritten Versuch wurde sowohl in der Reinsaat als auch im Gemenge Pferdemist und Kompost mit nahezu identischen N-Mengen, aber unterschiedlichen C-Mengen appliziert. Dieser Versuch hatte folgende Ziele: (1) die vorteilhaften Auswirkungen des Pferdemistes und des Komposts auf den Ertrag der Erbsen zu beurteilen, (2) zu untersuchen, ob diese Effekte durch die mikrobielle Besiedelung der Wurzeln, der

mikrobiellen Biomasse und der CO_2-Produktion erklärt werden können und (3) die Effekte der Rückstände der organischen Dünger auf den Ertrag der Folgefrüchte zu untersuchen. Die kurzzeitige Zugabe von Pferdemist und Kompost führte zu einer starken Stimulierung des mikrobiellen C, N und P sowie des pilzlichen Ergosterolgehaltes und der CO_2-Produktion, hatte jedoch keine Auswirkung auf den Ertrag der Pflanzen. Dies kann nicht allein auf einen N- und P-Mangel zurückgeführt werden, da in den gedüngten Pflanzen im Vergleich zur Kontrollbehandlung signifikant höhere Konzentrationen an N und P in den Körnern und im Stroh gemessen wurden. Weitere Ursachen können ebenso ein S-Mangel sowie andere Faktoren sein, die eine geringe Keimungsrate, ein schlechtes Auflaufen der Pflanzen und ein zu frühes Pflanzenwachstum hervorrufen. Es konnte festgestellt werden, dass die Rückstände der organischen Dünger (besonders der Pferdemist) den Ertrag der Folgefrucht (Winterweizen) positiv beeinflussen. Dies ist ein Hinweis auf signifikant positive Langzeiteffekte der langsamen Freisetzung von Nährstoffen aus dem Abbau der organischen Dünger. Im Gemenge konnte an Erbsenwurzeln im Vergleich mit Haferwurzeln eine höhere Mykorrhizierung sowie höhere Ergosterolgehalte festgestellt werden. Der Anbau von Mischkulturen ist ein wichtiges Instrument, um im ökologischen Landbau das Wachstum von Unkräutern auf Erbsenparzellen zu kontrollieren, beeinflusst aber weder die mikrobielle Besiedelung der Wurzeln, noch die Indikatoren der mikrobiellen Biomasse oder die CO_2-Freisetzung aus dem Boden. Durch die Hochrechnung der CO_2 Freisetzung in freigesetzte Menge pro Hektar konnte gezeigt werden, dass annähernd 40% des C aus dem Pferdemist und ca. 24% des Kompost-C während der 124 Tage zu CO_2 mineralisiert wurden. Weiterhin bestehen positiven Zusammenhänge zwischen Parametern der mikrobiellen Biomasse im Boden (C, N und P) und den N- und P-Konzentrationen im Pflanzenmaterial beider Spezies, die auf eine mögliche Verwendung der mikrobiellen Biomasse als Indikator für die Nährstoffverfügbarkeit für Pflanzen hinweisen.

Im vierten Experiment wurden Erbsen (*Pisum sativum* L.) und Hafer (*Avena sativa* L.) als Rein- oder Gemengesaat angebaut und jeweils mit 10 t C ha^{-1} Pferdemist und Grünkompost gedüngt. In diesem Versuch wurden mögliche positive Effekte der beiden organischen Dünger und der beiden Anbausysteme im ökologischen Landbau betrachtet. Im Speziellen wurde (a) der Ertrag der Erbsen- und Haferpflanzen bewertet, die als Rein- oder Gemengesaat angebaut wurden sowie deren N_2-Fixierung und Photosyntheserate, (b) der Ertrag von Weizen als Folgefrucht, (c) die mikrobiellen

Biomasse Indizes im Boden und in den Wurzeln und (d) die mikrobielle Aktivität untersucht. Die mikrobielle Aktivität wurde durch die CO_2- Freisetzung im Freiland und der als partikuläres organisches Material wiedergefundenen Mengen der organischen Dünger abgeschätzt. Es konnten positive Effekte der organischen Dünger auf den Ertrag der Erbsen festgestellt werden. Generell wurde durch die Zugabe der organischen Dünger die Menge des Trockengewichtes der Knöllchen, die Photosyntheserate, die N_2-Fixierung und die N-Akkumulation der Erbsen, sowie die N-Konzentration des Hafers verbessert. Im Erbse/Hafer-Gemenge ergab das Mittel der Düngevarianten eine Verringerung des Knöllchentrockengewichtes, der N_2-Fixierung und der Photosynthese der Erbsen um 14, 17 bzw. 12%. Ursächlich hierfür war die Beschattung des Haferwuchses. Außerdem wurde eine signifikante Erhöhung der Photosyntheserate des Hafers um 20% durch intraspezifische Konkurrenz zwischen den Haferpflanzen, erreicht. Das Flächenäquivalenzverhältnis (LER) der aus Erbsen und Hafer bestehenden Mischkultur überstieg 1.0, was auf einen Ertragsvorteil gegenüber der Reinkultur hinweist. Es wurden hoch signifikante Zusammenhänge zwischen dem Ertrag der Erbsen und der N-Aufnahme, der Knöllchenbildung, der N_2-Fixierung und der mittleren Photosyntheserate festgestellt ($r = 0,71 - 0,77$), was auf einen Beitrag dieser Indizes zur höheren Pflanzenproduktion hindeutet. Die mikrobielle Biomasse war sowohl in Rein- als auch in Mischkultur positiv mit den Trockenmasseerträgen der Erben korreliert. Die organischen Dünger steigerten die CO_2-Produktion und die C, N und P Gehalte der mikrobiellen Biomasse sowie das pilzliche Ergosterol im Boden. Die Anbausysteme hingegen hatten keine Effekte auf diese mikrobiellen Indizes. Aufgrund der Wiederfindung der organischen Dünger als POM konnte gezeigt werden, dass 70% (Mist) und 64% (Kompost) des zugegebenen C während der 101 Tage abgebaut wurden. Diesem konnten aber nur 39% (Mist) und 13% (Kompost) als CO_2-C zugeschrieben werden. Pferdemist war leichter für die Mikroorganismen im Boden verfügbar, was zu höheren Kornerträgen des nachfolgenden Winterweizens führte.

Im fünften Versuch wurden durch einen ferngesteuerten Hexakopter hoch aufgelöste True-Color Fotos aufgenommen und bodengestützte Messungen durchgeführt, um Behandlungseffekte des zweiten Feldversuchs zu beobachten. Die Bilder wurden zur Blüte 60 Tage nach der Aussaat, aufgenommen. Der Vegetationsindex „Normalized Green–Red Difference Index“ (NGRDI) wurde auf Grundlage der Luftbildaufnahmen berechnet und in Bezug zu Pflanzenparametern wie der oberirdischen Biomasse und des Blattflächenindex gesetzt (LAI). Die NGRDI

Werte lagen zwischen 0,09 und 0,13. Während keine signifikanten Unterschiede zwischen den Anbausystemen gefunden werden konnten, hatten die organischen Dünger einen signifikanten Einfluss auf den Pflanzenertrag und die entsprechenden NGRDI Werte. Die NGRDI Werte waren signifikant positiv mit der Trockenmasse korreliert (r = 0.58 - 0.78). Hohe Autokorrelation von NGRDI, und damit die Biomasse wurde zwischen die Parzellen festgestellt, die für Block-Kriging benutzt wurde, um die räumliche Variabilität im Feld zu zeigen. Für die Erbsen - und Haferpflanzen konnte kein Zusammenhang zwischen NGRDI und dem Blattflächenindex festgestellt werden. Die Aufnahme von Echtfarbfotos durch den ferngesteuerten Hexakopter und daraus erstellte NGRDI Werte stellen ein kosteneffizientes Verfahren zur Abschätzung der Biomasse und zur Entwicklung von Ertragsänderungskarten dar, um standortspezifische Empfehlungen geben zu können.

10. General conclusions and outlook:

1. No mycorrhizal infection was found in the roots of *myc*$^+$ peas at harvest. The used fertilizer rates were relatively high and probably inhibited mycorrhizal development. Plant biomass and grain production did not differ between the two pea isolines, with the exception of root weight, which was significantly higher in myc$^+$ peas. The lack of biological N_2 fixation in mutant P2 did not limit growth and yield. The incorporation of maize straw stimulates soil microbial biomass, but not the plant biomass. The recovery of non-decomposed residues by the sieving procedure indicated nearly complete decomposition of maize straw, both in the presence and absence of pea plants, but the decomposition was slightly retarded by the presence of living pea roots.

2. The comparison of the non-mycorrhizal mutant P2 and the symbiotic parental isoline Frisson makes it possible to investigate the additional effects of AMF and growing pea plants (*myc*$^+$ and *myc*$^-$) on the decomposition and microbial use efficiency of ^{15}N-labeled maize residues. Virtually nothing is known about the interactions of saprotrophic soil microorganisms, biotrophic AMF, and plant roots, despite the omnipresence of mycorrhizal symbiosis. The decomposition rate of added maize residues was significantly reduced by the presence of *myc*$^-$ peas, but especially by *myc*$^+$ peas, leading to a decreased turnover of the microbial biomass and an improved microbial substrate use efficiency. The formation of microbial residue C was increased and that of microbial residue N was reduced in the presence of plants. This means that the insufficient N supply to soil microorganisms and not a reduced water availability reduced the decomposition of maize residues in the presence of peas, especially *myc*$^+$ peas. AMF are apparently not involved in the decomposition of newly added organic residues, but intensify the competition between plants and soil microorganisms for available N. This finding needs support and further evidence by additional experiments.

In the both experiments, it was observed that the root biomass of myc$^-$ plants was clearly smaller compared with that of the myc$^+$ plants, in spite of no mycorrhizal colonization was detected in the first experiment. Kleikamp und Jörgensen (2006) have been mentioned that further genetic defects other than the lack of symbiosis cannot be completely excluded, which would limit the utilization of such mutant plants for the evaluation of AM symbiosis. The recovery of non-decomposed organic material indicated nearly complete decomposition of the added maize residues in both experiments. Moreover, the decomposition rate of added maize residues was significantly reduced by the presence of living pea roots.

3. Short term application of horse manure and compost greatly stimulated soil microbial biomass and CO_2 production, but failed to stimulate productivity of the current crops. Consequently, no correlation existed between any of the yield parameters and microbial biomass indices. In contrast, the close relationships between grain N and P concentrations and microbial biomass C, N and P suggest that the soil microbial biomass can be used as an indicator of nutrient availability to plants. Significant positive residual effects of organic fertilizer, especially horse manure were observed on the grain yield of the succeeding wheat. Mycorrhizal colonization and ergosterol content in roots differed significantly between the two crops, without any effects on yield. Intercropping is an important tool for controlling weeds on pea plots under organic farming conditions. Addition of organic manures one day before sowing, poor seedling emergence and microbial S immobilization appear to be the reasons for the absence of positive organic fertilizer effects on crop yield.

4. Application of C-rich organic fertilizers, such as yard-waste compost, but especially horse manure, greatly stimulated soil microbial biomass indices, which was reflected by increased pea yields in sole and intercropped systems. In contrast, compost and especially manure application did not enhance oat yields, due to the poor seedling emergence. The shading effect of the intercropped cereal component had adverse effects on nodulation, N_2 fixation, photosynthetic rates and biomass of the intercropped legume component. However the LER values showed that intercropped plants used growth resources on average 10-20% more efficiently. According to the organic fertilizer recovered as POM as well as the CO_2 production, horse manure was more readily available to soil microorganisms than compost, leading to increased grain yields of the succeeding winter wheat. Organic fertilization and legume/cereal intercropping are important means for improving soil fertility, not only in organic farming systems, which demands more scientific and practical attention

As shown from the field experiments, the horse manure clearly decreased the grain yield and straw of oats in sole and intercropping oats due to the poor seedling emergence. In contrast to the first field experiment, where organic fertilizers were applied one day before sowing and less well mixed into the soil, pea plants in the second experiment, responded significantly to applied organic fertilizers in terms of nodule mass, N_2 fixation and photosynthesis, both in sole and intercrop systems, indicating favorable growth conditions. In the both field experiments the following results were observed (1) the positive effect of organic fertlizer on the microbial parameters and microbial

activity. (2) AMF colonization was obviously stronger in pea roots than in oats. (3) Ergosterol concentration was distinctly higher in pea than in oat roots and increased dramatically during maturation. (4) Intercropping is an important tool for controlling weeds on pea plots. (5) Intercropping did not affect microbial root colonization, soil microbial biomass indices or CO_2 evolution from the soil surface. (6) Positive residual effects of organic fertilizers on the succeeding winter wheat.

The addition of C-rich organic fertilizer improved the microbial biomass and their activity, which was reflected on increased plant nutrient and biomass. The striking feature of the present field data was the relationship found between the microbial biomass indices in soil and nutrient concentration in plant tissues and peas biomass, indicating the important role of soil microorganisms in plant nutrition. C-rich organic fertilizer has shown a positive effect on the N_2 Fixation in peas, which is considered an important factor in organic farming systems.

5. The small low-altitude hexacopter is a rapid, simple and cheap technique for acquiring high resolution images to be used in precision agriculture. The observed correlation between NGRDI and aboveground biomass indicates that true color images allow to determine crop biomass and to establish yield variation maps of an entire field. The fit between the spatial distribution of the NGRDI and the total aboveground biomass has been shown to be a critical factor in precision agriculture. The calculated VI, not including NIR information, failed to detect differences between cropping systems and was not correlated with LAI. The current results are encouraging for the development of UAVs as a tool for site-specific precision agriculture in a small field area. For the adoption of this technique to evaluate the yield and others plant parameters, additional experiments are needed with several measuring dates for several species.

11. Supplementary material

Supplementary-Table 1: Some yield components of pea and oat at 120 DAS (senescence stage of pea, BBCH 97 and late hard dough stage of oat BBCH 87-89) in sole peas and in peas intercropped with oats; different letters within a column indicate a significant difference (LSD-test, $P < 0.05$).

	Pods or spikes ($n\ m^{-2}$)	100 or 1000-seed weight (g)	Harvest index (%)
Peas			
Sole peas	336	22.6	54
Sole peas + manure	288	21.0	52
Sole peas + compost	235	22.4	50
Intercropped peas	198	19.9	46
Intercropped peas + manure	228	19.9	49
Intercropped peas + compost	201	18.8	45
Probability values			
Cropping system	0.01	<0.01	0.01
Fertilizer	NS	NS	NS
System × fertilizer	NS	NS	NS
CV (±%)	21	10	8
Oats			
Intercropped peas	130 a	40.1 a	52 a
Intercropped peas + manure	130 a	41.9 a	50 a
Intercropped peas + compost	129 a	41.3 a	52 a
CV (±%)	16	8	5

CV = mean coefficient of variation between replicate plots (n = 4), NS = not significant.

Supplementary-Table 2: Some growth and yield components per plant in sole peas and in peas intercropped with oats; nodulation was determined at late flowering stage (60 DAS, BBCH 69), other components were determined at senescence stage (120 DAS, BBCH 97).

	Nodules		Height	Dry weight	Pods	Grain
	(n plant^{-1})	(mg plant^{-1})	(cm)	(g plant^{-1})	(n plant^{-1})	
Sole peas	20	9.0	73	11.4	7.6	26.8
Sole peas + manure	19	8.1	72	9.3	6.7	22.5
Sole peas + compost	24	9.7	75	13.4	8.0	30.8
Intercropped peas	17	7.3	74	8.2	5.8	19.6
Intercropped peas + manure	13	5.2	67	8.4	5.8	20.8
Intercropped peas+ compost	16	7.7	71	7.2	5.4	16.4
Probability values						
Cropping system	<0.01	0.02	NS	<0.01	<0.01	0.01
Fertilizer	NS	NS	NS	NS	NS	NS
Crop × fertilizer	NS	NS	NS	NS	NS	0.04
CV (±%)	40	46	10	32	27	38

CV = mean coefficient of variation between replicate plots (n = 12), NS = not significant.

Supplementary Table 3: Some yield components of pea and oat at 94 DAS under three cropping systems: sole pea, pea/oat intercrop and sole oat with three fertilizer treatments: no fertilizer, horse manure and compost.

Treatment	Pod number ($n\ m^{-2}$)	100-seed weight (g)	Spike number ($n\ m^{-2}$)	1000-seed weight (g)
Sole pea	384	23.3		
Sole pea + manure	439	22.7		
Sole pea + compost	446	22.8		
Pea/oat intercrop	201	18.0	157	32
Pea/oat intercrop + manure	314	19.4	123	34
Pea/oat intercrop + compost	216	18.0	159	33
Sole oat			349	33
Sole oat + manure			382	35
Sole oat + compost			380	35
Probability values				
Cropping system	<0.01	<0.01	<0.01	<0.01
Fertilizer	<0.01	<0.01	<0.01	0.01
System x fertilizer	<0.01	0.03	0.01	0.03
CV (±%)	11	3	8	3

CV = coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing

Supplementary Table 4: Some growth parameters per plant of pea at 62 DAS under three cropping systems: sole pea, pea/oat intercrop and sole oat with three fertilizer treatments: no fertilizer, horse manure and compost.

Treatment	Nodules		Leaflet	Leaf area	Leaf area
	(n plant^{-1})	(mg plant^{-1})	(n plant^{-1})	(cm^2 plant^{-1})	index
Sole pea	39	21.4	23.6	223	2.2
Sole pea + manure	57	37.3	25.1	270	2.6
Sole pea + compost	42	25.6	21.4	213	2.1
Pea/oat intercrop	42	24.9	22.5	187	1.5
Pea/oat intercrop + manure	38	24.6	24.3	223	1.8
Pea/oat intercrop + compost	30	14.2	22.9	214	1.7
Probability values					
Cropping system	<0.01	<0.01	NS	0.04	<0.001
Fertilizer	<0.01	<0.01	<0.01	0.03	0.08
System x fertilizer	0.01	<0.01	NS	NS	NS
CV (±%)	43	47	10	24	36

CV = coefficient of variation between replicate plots (n = 29-45 for nodulation and n = 12 for other growth parameters), NS = not significant, DAS = days after sowing

Supplementary Table 5: Some yield components per plant of pea and oat at 94 DAS under three cropping systems: sole pea, pea/oat intercrop and sole oat with three fertilizer treatments: no fertilizer, horse manure and compost.

Treatment	Plant DW (g)	Pod or spike (n. plant^{-1})	Grain (n. plant^{-1})
Pea			
Sole pea	9.8	7.0	22.1
Sole pea + manure	13.0	8.5	30.4
Sole pea + compost	10.0	7.2	23.4
Pea/oat intercrop	5.0	3.6	11.6
Pea/oat intercrop + manure	8.9	6.1	21.0
Pea/oat intercrop + compost	5.6	4.2	14.3
Probability values			
Cropping system	<0.001	<0.001	<0.001
Fertilizer	<0.001	<0.001	<0.001
System x fertilizer	NS	NS	NS
CV (±%)	27	27	29
Oat			
Pea/oat intercrop	27.7	3.7	281
Pea/oat intercrop + manure	30.5	4.1	246
Pea/oat intercrop + compost	28.9	4.0	269
Sole oat	19.9	3.3	206
Sole oat + manure	23.3	3.9	241
Sole oat + compost	21.2	3.4	216
Probability values			
Cropping system	<0.001	0.03	<0.01
Fertilizer	NS	NS	NS
System x fertilizer	NS	NS	NS
CV (±%)	21	6	28

CV = coefficient of variation between replicate plots (n = 20), NS = not significant, DAS= days after sowing

Supplementary Table 6a: Organic C recovered in the two fractions of particulate organic matter (POM) 0.4-2 and >2 mm after 19, 67 and 101 DAS.

Treatment	POM-C (mg g^{-1} soil)					
	19 DAS		67 DAS		101 DAS	
	0.4-2 mm	>2 mm	0.4-2 mm	>2 mm	0.4-2 mm	>2 mm
Sole pea	0.21	0.10	0.13	0.08	0.22	0.20
Sole pea + manure	0.85	2.39	0.68	1.33	0.71	1.05
Sole pea + compost	2.20	1.04	1.68	1.02	1.34	0.97
Pea/oat intercrop	0.18	0.12	0.16	0.07	0.19	0.17
Pea/oat intercrop + manure	0.82	2.82	0.80	1.42	0.66	1.17
Pea/oat intercrop + compost	2.05	1.19	1.77	0.87	1.28	0.72
Sole oat	0.20	0.10	0.16	0.09	0.18	0.12
Sole oat + manure	0.89	2.40	0.78	1.81	0.71	1.25
Sole oat + copmost	2.12	1.06	1.91	1.01	1.18	0.88
Probability values						
Cropping system	NS	NS	NS	0.02	NS	NS
Fertilizer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
System x fertilizer	NS	NS	NS	NS	NS	NS
CV (±%)	13	23	24	21	20	25

CV = coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing

Supplementary -Table 6b: Organic N recovered in the two fractions of particulate organic matter (POM) 0.4-2 and >2 mm after 19, 67 and 101 DAS

Treatment	POM-N (µg g^{-1} soil)					
	19 DAS		67 DAS		101 DAS	
	0.4-2 mm	>2 mm	0.4-2 mm	>2 mm	0.4-2 mm	>2 mm
Sole pea	9.2	3.2	7.6	3.4	13.9	8.2
Sole pea + manure	33.4	65.0	33.7	46.6	37.4	41.5
Sole pea + compost	110.0	40.8	90.1	38.0	76.7	46.4
Pea/oat intercrop	9.3	3.6	8.9	2.4	11.0	6.0
Pea/oat intercrop + manure	35.4	79.3	41.0	50.8	35.1	45.5
Pea/oat intercrop + compost	101.3	41.7	95.2	29.4	71.6	33.1
Sole oat	9.9	2.9	8.8	3.1	9.9	4.1
Sole oat + manure	35.8	69.2	36.1	64.3	35.8	51.6
Sole oat + compost	114.7	36.6	104.5	36.9	66.2	38.2
Probability values						
Cropping system	NS	NS	NS	0.02	NS	NS
Fertilizer	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
System x fertilizer	NS	NS	NS	NS	NS	0.03
CV (±%)	10	24	24	21	20	26

CV = coefficient of variation between replicate plots (n = 4), NS = not significant, DAS = days after sowing

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