

# **On Soil Organic Matter Dynamics in Species-Diverse Grasslands and Intercrop Systems**

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# **On Soil Organic Matter Dynamics in Species-Diverse Grasslands and Intercrop Systems**

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## **Thesis**

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*To my beloved wife Jingying Jing*



## Abstract

Natural and agricultural plant mixtures generally have greater biomass production than monocultures. Effects of plant diversity on carbon sequestration in soil are therefore plausible but have been little explored. This thesis aims to examine the effect of plant species diversity on SOM dynamics in species-diverse grasslands and intercrop systems.

It was found that plant species diversity promoted soil carbon (C) and nitrogen (N) storage in an 11-year grassland biodiversity experiment and a 7-year intercropping experiment. Above- and below-ground overyielding in more diverse systems provide a plausible explanation for greater soil C storage. Analyses of soil  $\delta^{15}\text{N}$  indicated that biological N fixation contributed to increased soil N storage in legume-based intercrops. Furthermore, increases in N storage in grasslands and intercrops without legumes indicated a contribution by reduced N losses. Decomposition experiments conducted in the laboratory and in the field showed that plant species diversity increased the relative decomposition rate of SOM in both systems. This acceleration effect was attributed to changes in SOM quality, resulting from a greater proportion of labile C in soil caused by enhanced below-ground biomass production. Moreover, plant species diversity increased the decomposition of fresh root litter. This increase was predominantly driven by a soil-mediated effect, rather than a litter-mixing effect on substrate quality.

Overall, this thesis shows that the positive effects of plant species diversity on several ecosystem functions (i.e. soil C and N storage, biomass production and SOM decomposition) reported for perennial N-limited grasslands are paralleled in annual N-rich intercropping agroecosystems. These findings suggest that greater plant species diversity can improve ecosystem service provisioning by simultaneously enhancing biomass production, improving soil fertility and mitigating greenhouse gas emissions.





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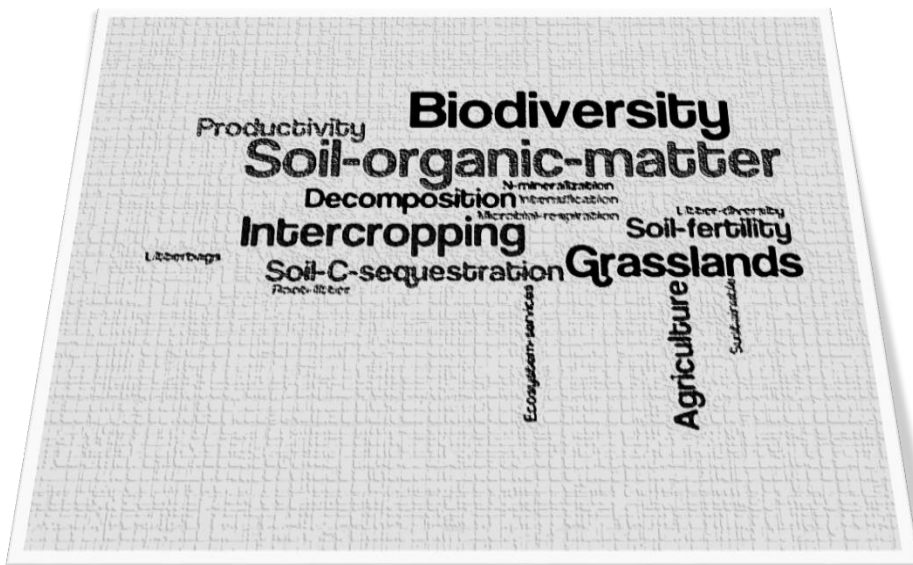
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# Abbreviations

ANOVA	Analysis of Variance
CCD	Cumulative Carbon Decomposed
LER	Land Equivalent Ratio
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
STN	Soil Total Nitrogen

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## General introduction





## Relevance

Globally, more than two-thirds of terrestrial carbon (C) is stored in soils, with a pool size more than twice that of the total C in the atmosphere (Schimel, 1995). Thus, changes in soil C stocks have an important impact on the global C cycle (Raich and Potter, 1995). Due to human land-use activities, such as the conversion of natural vegetation to agricultural land, soil organic C has declined by 10%-59% (Guo and Gifford, 2002), resulting in approximately 35% of historical anthropogenic carbon dioxide (CO<sub>2</sub>) emissions (1850-1990) (Foley et al., 2005), thereby contributing to global warming and related environmental problems. Carbon and nitrogen (N) are the main components of soil organic matter (SOM), which plays an essential role in determining soil fertility and providing nutrients for plant growth (Troeh and Thompson, 2005). Reduced soil fertility due to depletion of SOM is imposing an unprecedented challenge for our rising food demand in the context of a rapidly growing human population (Sanchez, 2002). Thus, increasing soil organic C sequestration in soil is a key way to mitigate climate change, enhance soil fertility and thus sustain food production (Lal, 2004).

Confronting the dual challenges of global warming related to rising atmospheric CO<sub>2</sub> concentrations (IPCC, 2001) and loss of plant species (Wilson, 1999), there is an increasing interest in exploring the links between plant species diversity and soil C sequestration. For example, a few grassland biodiversity experiments have shown positive effects of plant diversity on soil C sequestration (Tilman et al., 2006; Fornara and Tilman, 2008; Steinbeiss et al., 2008). Furthermore, a large number of studies have shown that plant species diversity generally enhances productivity (Hooper et al., 2005; Cardinale et al., 2012). These findings imply that more C may be sequestered in soils as a consequence of enhanced biomass production in diversified plant systems (Catovsky et al., 2002). Moreover, plant diversity has been found to promote N accumulation in soil (Fornara and Tilman, 2008). The increases in soil C and N could be attributed to the presence of legumes, which can access atmospheric N via symbiotic N fixation by rhizobium. As most grasslands are N-limited, the presence of legumes can strongly enhance N input into such systems, thereby increasing plant productivity and C sequestration into soil. However, it remains to be established whether plant species diversity can enhance soil C

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and N storage in the absence of legumes, and which mechanisms, if any, may drive this effect.

Crop diversification within agroecosystems is being increasingly promoted as a plausible strategy for sustainable agriculture (Matson et al., 1997; Tilman et al., 2002; Bommarco et al., 2013). Intercropping, an agricultural practice in which two or more crop species or varieties are cultivated simultaneously on the same piece of land (Vandermeer, 1989), is one of the approaches to increase plant diversity in agroecosystems. A large body of literature has reported the advantages of intercropping in terms of enhanced crop production, reduced pest and disease pressure, and mitigated risks of complete crop failure (Liebman and Dyck, 1993; Trenbath, 1993; Zhu et al., 2000; Rusinamhodzi et al., 2012). However, little is known regarding the effect of intercropping on soil C and N storage, or the underlying mechanisms controlling soil C and N dynamics. Understanding such effects and mechanisms is crucial for strengthening the ecological foundations of sustainable agriculture because the accumulation of soil C and N can improve soil fertility and benefit plant productivity over the long term.

This thesis aims to examine and compare the effect of plant species diversity on SOM dynamics in grassland communities without legumes, and in intercropping agroecosystems. The next section will first describe SOM dynamics and then review how such dynamics may respond to changes in plant species diversity in the two contrasting systems.

## Literature Review

### ***Soil organic matter dynamics***

Soil organic matter is defined as a mixture of all plant material in various stages of decomposition, microbial biomass as well as stable organic matter (Stevenson, 1994). Soil organic C (SOC) is the C fraction of these pools of organic matter. Soil C levels are ultimately determined by the balance between the C input, for example via belowground allocation of photosynthates, and the C losses such as decomposition (Amundson, 2001). Because of human disturbances, such as increased frequency of fire events in natural

systems (Schlesinger and Bernhardt, 2013), or the rising demand for food and biofuel from agroecosystems, soil C input from aboveground litter or crop residues may be further reduced (Lal, 2005). As such, belowground C inputs are likely to predominantly contribute to the SOC accumulation in these ecosystems. These C inputs consist of structural (commonly thick) roots, fine roots ( $< 1\text{ mm}$ ) and rhizodeposits released by plants which represent belowground productivity. Since fine roots and rhizodeposits are commonly short-lived, especially the latter (Kuzakov and Domanski, 2000), such inputs are less likely to leave a long-term legacy in the soil when compared to structural roots composed of more recalcitrant substances. Combined with this, the methods currently used for determining fine root production and rhizodeposition have been criticized (Hendricks et al., 2006) and are especially difficult to execute under mixed species conditions in the field. Therefore, in this thesis, I focus on coarse ( $> 1\text{ mm}$ ) root biomass as a proxy for belowground C input.

Decomposition is generally defined as the process through which organic matter is broken down. During the process, recalcitrant organic compounds are formed,  $\text{CO}_2$  is released back to atmosphere, and nutrients are mobilised or incorporated by soil microbes. Decomposition of fresh litter and SOM in terrestrial ecosystems plays a key role in the carbon balance of soils and the cycling of nutrients for plant production. Decomposition process is mainly regulated by three driving factors: resource quality, physico-chemical environment and decomposer organisms. The latter two are also collectively referred to as decomposition microenvironment. In this thesis, I study the decomposition dynamics of fresh root litter and SOM as two pathways for soil C losses in a short term (less than 1 year), and explore which factors drive the decomposition dynamics. There are factors which control long-term decomposition rate of organic matter. These include organic matter being stabilized against decomposition through physical, chemical and biochemical protection of organic matter (Cadisch and Giller, 1997; Hassink, 1997; Six et al., 2002). However, such factors are not studied in this thesis.

### ***Soil organic matter dynamics in natural grasslands***

Since late 1990s, several grassland biodiversity experiments have been conducted to examine the effects of plant species diversity on vegetation productivity (Loreau et al.,

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2001; Hooper et al., 2005; Cardinale et al., 2012). The general pattern emerging from these studies is that productivity (both above-ground and below-ground) increases with species diversity. However, the underlying mechanisms have been hotly debated (Hooper et al., 2005). One controversy is whether the observed biomass increase should be attributed to niche complementarity (Tilman et al., 1997b) or a selection effect (Huston, 1997). The former refers to complementary use of resources (such as N between legumes and non-legumes), leading to more complete acquisition of resources, or facilitative interactions among species (such as an alleviation of harsh environmental conditions by other species). Conversely, the selection effect is generated by chance: more diverse mixtures have a greater chance of including a highly productive species. Another debate involves the role of legumes. Legumes can obtain additional atmospheric N via a symbiosis with *Rhizobium* bacteria. The fixed N can then be translocated to co-existing plant species via root exudation, root turnover and common mycorrhizal networks (Hogh-Jensen and Schjoerring, 2000; Van der Heijden and Horton, 2009). Thus, legumes can enhance N input into soil and substantially contribute to the increase in plant productivity especially in N-limited grasslands. Recent studies show that plant species richness increases productivity in the absence of legumes (Van Ruijven and Berendse, 2003; Van Ruijven and Berendse, 2005). This raises the question as to whether plant species richness can enhanced soil C and N storage through enhanced productivity in the absence of legumes.

While the effects of plant species richness on productivity have been widely studied (Loreau et al., 2001; Hooper et al., 2005; Cardinale et al., 2012), there has been far less work on decomposition of organic matter. To date, studies investigating the relationship between plant species richness and decomposition have focused on litter decomposition. The results are not straightforward. On the one hand, studies manipulating the diversity of litter have explored whether plant species richness can affect litter decomposition through changes in litter diversity. They show that there is no consistent effect of litter diversity on litter decomposition, but non-additive litter mixing effects prevail in these studies (Wardle et al., 1997; Mikola et al., 2002; Gartner and Cardon, 2004; Hattenschwiler et al., 2005). In other words, mixing litter decomposed differently than expected from the decomposition of single litters. This phenomenon is commonly



observed when the components of litter mixture vary in resource quality (Wardle et al., 1997). On the other hand, studies based upon grassland biodiversity experiments have investigated whether plant species richness can influence litter decomposition through changes in the decomposition microenvironment. Some studies show that decomposition rate of standard litter increased with species richness (Hector et al., 2000; Knops et al., 2001; Spehn et al., 2005; Vogel et al., 2013), whereas there was no relationship between plant species richness and decomposition at the Swiss and German sites of the BIODDEPTH project (Spehn et al., 2000; Scherer-Lorenzen, 2008). While previous studies have examined the two potential pathways (i.e. litter mixing effect and soil ecosystem effect) independently, it is essential to compare their relative impacts on litter decomposition in one study and assess whether there are interactions. Furthermore, these decomposition studies focus on leaf litter; it remains to be established whether these effects also occur for root litter.

Accumulation of C in soil is generally a slow process and few biodiversity experiments are of sufficient duration to explore soil C dynamics and underlying mechanisms (but see De Deyn et al., 2009). To date, only a few studies have examined the effect of plant species richness on SOC decomposition. Dijkstra et al. (2005) and Reid et al. (2012) found that the relative decomposition rate of SOC increased with plant species richness and suggested the positive species richness effect was largely due to the enhanced biomass production associated with increased species richness. It is probable that the larger fresh organic matter inputs into soil may have led to greater proportion of labile C in soil, thereby accelerating the rate of decomposition. In addition, recent studies have suggested that plant species richness can enhance the rate of decomposition of SOC through providing a diversity of plant-derived resources, and stimulating more diverse decomposer communities and enzymatic activity (Stephan et al., 2000; Meier and Bowman, 2008; Eisenhauer et al., 2011).

To date, only a few studies have investigated the relationships between plant species richness and soil C storage. The “Cedar Creek Biodiversity Experiment” (BioDIV) in USA found a positive effect of plant species richness on soil C storage after 12 years (Fornara and Tilman, 2008). The “Jena” Experiment in Germany demonstrated that plant

diversity promotes short-term soil C storage after 4 years (Steinbeiss et al., 2008). Both studies attributed the positive species richness effect to enhanced root biomass production (hence increased soil C inputs) associated with plant species richness. In contrast, soil C storage did not change in response to plant diversity in another 9-year biodiversity experiment in Cedar Creek, the “Biodiversity, CO<sub>2</sub>, and N Experiment” (BioCON) (Reid et al., 2012). This study suggested that the absence of a plant species richness effect on soil C storage was due to the increased rate of SOM decomposition in high species richness mixtures which counteracted the effects of enhanced plant productivity (Reich et al., 2012). Collectively, these studies suggest that the dynamics of soil C storage are determined by the relative impacts of plant species richness on plant productivity versus on SOC decomposition.

Further to soil C storage and dynamics, recent studies have shown that plant species richness can enhance soil fertility, indicated by enhanced soil N storage (Fornara and Tilman, 2008) and soil N mineralization (Dybziński et al., 2008; Oelmann et al., 2011). Enhanced soil fertility can feedback positively to plant productivity. This may contribute a possible explanation for the strengthening of the diversity-productivity relationship over time, reported in several long-term biodiversity experiments (Cardinale et al., 2007; Van Ruijven and Berendse, 2009; Allan et al., 2011; Reich et al., 2012). Increasing plant productivity over time could further increase soil C and N storage in the long term. Legumes have also been shown to promote soil C and N accumulation (Fornara and Tilman, 2008). As more diverse plant mixtures have an increased chance of including legumes, whether plant diversity can enhance soil C and N storage in the absence of legumes is still an open question.

### ***Soil organic matter dynamics in intercropping agroecosystems***

A question which remains to be answered is whether the same patterns and underlying mechanisms that have been observed in natural grasslands still hold true in diversified agroecosystems? The answer is not self-evident because agroecosystems differ in many aspects from natural grasslands. First, agroecosystems consist mainly of annual species that are cultivated in rotation, while grasslands are dominated by perennials. The benefits of plant diversity for suppressing soil pathogens observed in natural grasslands (De

Kroon et al., 2012) may be counteracted by crop rotation in diversified agricultural systems (Peters et al., 2003). Second, roots of annuals usually decompose faster than those of perennials because of comparatively higher litter N content (Wardle et al., 1997). Third, aboveground residues are often removed for other uses (e.g. biofuel) in agroecosystems, reducing litter input into the soil. Finally, the role of biological N fixation of legumes is suppressed in high-N input agricultural systems (Salvagiotti et al., 2008). Therefore, it is impossible to extrapolate the findings from natural grasslands to agroecosystems.

Intercropping is an ancient agricultural practice involving two or more crop species or genotypes, growing together and co-existing for a period of time (Vandermeer, 1989). As the definition indicates, intercropping can be divided into several types, varying in time and space to some degree (Lithourgidis et al., 2011). These types include mixed intercropping (i.e. simultaneously growing two or more crops with no distinct arrangement), strip intercropping (i.e. growing two or more crops in alternate strips) and relay intercropping (i.e. growing a second crop before the first crop is mature), with the latter two being most commonly practiced by farmers. Moreover, intercropping can combine crop species from different functional groups. The most typical combinations are cereal/cereal and cereal/legume intercropping. In this thesis, I focus on the two intercropping types.

Intercropping is important not only in subsistence or low-input agricultural systems, but in high-input and high-yielding systems as well (Vandermeer, 1989; Lithourgidis et al., 2011; Li et al., 2013). The most obvious advantage of intercropping is that it can produce larger crop yields than monocropping per unit of land, often characterized by land equivalent ratios (LER) above 1. LER is a common measure to express a yield advantage in intercrops. LER greater than 1 means that more than 1 ha of land is needed to produce the same yield in monocrops as 1 ha of intercrops (Mead and Willey, 1980). Increased crop yields are attributed to complementary temporal or spatial acquisition of resources, such as light, water and nutrients (Hinsinger et al., 2011) or beneficial neighbour interactions (facilitation) (Zhang and Li, 2003; Hauggaard-Nielsen and Jensen, 2005; Li et al., 2007), and/or reduction in the impacts of weeds, pests and diseases (Liebman and Dyck, 1993; Trenbath, 1993; Zhu et al., 2000). A classic example is the “Three Sisters”

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system where maize, beans and squash are grown together (Lewandowski, 1987). Squash acts as ground cover during the early season, suppressing the growth of early-season weeds and reducing water loss by evaporation. Maize and beans maximize the utilization of soil mineral N and biologically fixed N during the later season (Jensen, 1996b). Moreover, the fixed N by legumes can be transferred to maize via root exudation (Xiao et al., 2004). Furthermore, differences in root architecture among the three crops (shallow-taproot bean, deep-fibrous root maize and deep-taproot squash) allow them to efficiently utilize soil nutrients in the whole profile (Postma and Lynch, 2012). These mechanisms also apply to cereal/cereal and cereal/legume intercrops in high-N input systems (Li et al., 2005; Li et al., 2006; Li et al., 2009). Collectively, these mechanisms may contribute to greater crop productivity and enhanced N input (e.g. cereal/legume combination) or increased N retention (e.g. cereal/cereal combination) in intercropping systems.

Although aboveground overyielding in intercrops has been extensively demonstrated (Zhang and Li, 2003; Lithourgidis et al., 2011), it is unclear whether aboveground overyielding is mirrored belowground; most experiments have focused on crop yields or aboveground biomass only. Intercropping systems that consist of species with differing growing periods can intercept more light than single cropping systems, due to having vegetation cover for longer portions of each year. This results in greater aboveground biomass and yield. This increase is likely to also result in greater root biomass as crop species commonly maintain a functional balance between the shoot and the root system (Poorter et al., 2012). To date, only a few studies have explored the effect of intercropping on root weight density and root biomass of component species (Ghosh et al., 2006; Yang et al., 2010; Li et al., 2011b). For example, Ghosh et al. (2006) showed that intercropping increased root biomass of sorghum but decreased that of soybean compared to their respective monocrops. However, the link to belowground productivity in intercropping systems remains to be firmly established.

Increased biomass production from intercropping may not result in increased soil C storage if decomposition is also enhanced. Little is known about the effect of intercropping on the decomposition of organic matter. A recent study explored the effect of mixing crop residues on residue decomposition and showed that the low-N residue of

maize decomposed faster when mixed with high-N soybean residues (Vachon and Oelbermann, 2011). Intercropping may also influence organic matter decomposition via changes in the decomposition microenvironment, such as soil nutrients levels (Li et al., 2005) or soil microbial biomass and microbial community composition (Song et al., 2007; Wang et al., 2007; He et al., 2013). In addition to the decomposition of crop residues, only one study compared decomposition rate of SOC in monocropping and intercropping soils (Rusinamhodzi et al., 2009), finding no significant differences. More research is needed to systematically understand how intercropping can affect the decomposition of crop residue as well as SOC dynamics.

Recently, a few studies have attempted to explore the effect of intercropping on soil C and N storage (Myaka et al., 2006; Snapp et al., 2010; Oelbermann and Echarte, 2011). However, the duration of these studies was too short to convincingly demonstrate sequestration of soil C or N. A recent 2-year study reported that maize/soybean intercropping resulted in a slightly higher soil C content than soybean monocrop, but the C content did not differ from maize monocrop (Dyer et al., 2012). The experimental design of this study did not follow a substitutive design, introducing confounding effects between diversity and density variables. Thus, it is still unclear whether increased diversity through intercropping can enhance soil C and N storage.

## Objectives

Given the above research needs, the main objective of this thesis is to increase our understanding of plant species richness on SOM dynamics in both species-diverse grasslands and intercrop systems. The specific objectives are:

1. To investigate the effects of plant species richness on soil C and N storage in grasslands without legumes and to reveal the mechanisms underlying the soil C change from the perspective of plant productivity and SOC decomposition (Chapter 2).
2. To explore and disentangle the effects of plant species richness in grasslands on the decomposition of root litter via changes in litter mixing and changes in the decomposition microenvironment (Chapter 3).

3. To determine the effects of intercropping on soil C and N storage and reveal the underlying mechanisms from the perspective of belowground productivity (Chapter 4).
4. To explore and disentangle the effects of intercropping on the decomposition of root litter and SOC via changes in litter mixing and changes in the decomposition microenvironment (Chapter 5).

## Two long-term field experiments

To address the above research objectives, I utilised two long-term field experiments as described below throughout this thesis. They provided the opportunity to explore the effects of plant species diversity on soil C and N dynamics in grassland ecosystems and intercropping agroecosystems.

### ***Wageningen grassland biodiversity experiment***

The Wageningen grassland biodiversity experiment was established on a former arable field in Wageningen, the Netherlands, in 2000 (Fig. 1.1A). It is unique in the world because it is the only long-term grassland biodiversity experiment that excludes legumes. In contrast, the two Cedar Creek biodiversity experiments (“BioDIV” and “BioCON”) in Minnesota, USA and the Jena biodiversity experiment in Germany all include legumes. Briefly, the Wageningen biodiversity experiment comprised 102 plots of 1 m<sup>2</sup>, arranged in a randomized block design with four levels of species richness (monocultures, two-species, four-species, and eight-species). The species pool comprised four perennial grass and four perennial forb species, which commonly coexist in European hay meadows. Detailed information is reported in Van Ruijven and Berendse (2003). Previous findings from the experiment are that aboveground biomass increased with plant species richness, and the positive diversity effect was driven by a niche complementarity effect rather than a selection effect (Van Ruijven and Berendse, 2003). Moreover, the positive effects of species diversity on productivity strengthened in the first four years but remained constant thereafter, suggesting a long-term persistence of this diversity-productivity relationship (Van Ruijven and Berendse, 2005; Van Ruijven and Berendse, 2009). However, no information on soil C and N storage has been presented to date.

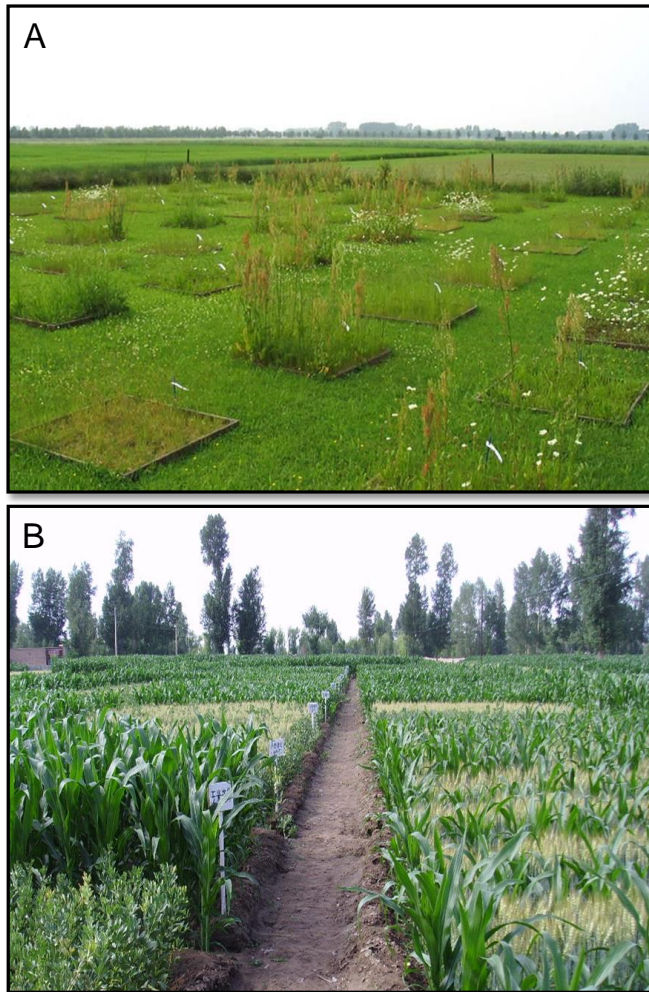


Figure 1.1 The Wageningen grassland biodiversity experiment (A) and the Wuwei intercropping experiment (B). Photo (A) is from Jasper van Ruijven.

### ***Wuwei intercropping experiment***

The Wuwei intercropping experiment, initiated in 2003 (Fig. 1.1B), is the oldest one of its kind in existence today. It consists of three important and functionally diverse crop species: wheat (cereal, C3), maize (cereal, C4) and faba bean (legume, C3). Crops were arranged in four commonly practiced cropping systems: continuous monocropping, rotation, continuous intercropping (with each crop cultivated yearly in the same place) and rotational intercropping (with each crop rotated to the other crop's strips each year).

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This led to three functionally complementary combinations of crops (i.e. wheat and maize; wheat and faba bean; maize and faba bean) in rotation and intercropping systems. The experimental design was a completely randomized block design with twelve cropping systems and three blocks. A substitutive design was used to avoid confounding plant density effects. In this experiment, I studied the effect of intercropping on soil C and N dynamics, as well as the role of functional complementarity among species on such dynamics. Previous studies from this experiment showed that intercropping significantly enhanced grain yields and aboveground biomass through complementary or facilitated resource use (Li et al., 2007). In addition, it was suggested that intercropping of crop species that vary in phenology may improve crop N use efficiency and reduce inorganic N accumulation in soil (Li et al., 2005).

## Outline of the thesis

This thesis consists of six chapters, including the General Introduction. The four objectives stated above correspond with the following four chapters:

In **Chapter 2**, I determine the effects of plant species richness on soil C and N storage based on the Wageningen grassland biodiversity experiment. Aboveground biomass over the 11 years, standing root biomass in the 10<sup>th</sup> year and SOC decomposition were also measured to evaluate the relative impacts of species richness on soil C inputs and C losses in order to explain the change in SOC. In addition, soil N mineralization was determined to link the feedback of SOM change to plant productivity.

In **Chapter 3**, I further explore the effects of species richness on root litter decomposition. A laboratory incubation experiment was conducted to disentangle the diversity-induced effects into two potential pathways: changes in litter diversity and changes in the decomposition microenvironment.

In **Chapter 4**, I investigate the effects of intercropping on soil C and N storage in the Wuwei intercropping experiment. Two short-term field experiments were conducted to determine peak standing root biomass using a monolith method (Böhm, 1979) to unravel the mechanisms underlying the changes in SOC and soil N.



In **Chapter 5**, I further explore the mechanisms underlying the changes in SOC through studying intercropping effects on decomposition of SOC and root litter. Using the same approach as in Chapter 3, both laboratory incubation and field litterbag experiments were conducted to separate the effect of litter diversity from that of the decomposition microenvironment on root litter decomposition. A laboratory incubation experiment with and without a standard substrate was used to investigate intercropping effects on SOC decomposition and whether this effect is driven by changes in the decomposition microenvironment. In addition, two intercropping combinations (wheat/maize and maize/faba bean) were chosen to examine whether these effects depend on the presence of legumes.

The thesis finishes with the General Discussion, in which the effects and mechanisms of plant species diversity in two contrasting ecosystems are compared and synthesized. Moreover, I estimate soil C sequestration potential of species diversity in the two ecosystems and address the implications for long-term soil fertility.



## Chapter 2

### **Plant species richness promotes soil carbon and nitrogen storage in grasslands without legumes**

*Wenfeng Cong, Jasper van Ruijven, Liesje Mommer,  
Gerlinde B. De Deyn, Frank Berendse and Ellis Hoffland*



*Photo by Jasper van Ruijven*

### **Abstract**

The storage of carbon (C) and nitrogen (N) in soil are important ecosystem functions. Grassland biodiversity experiments have shown a positive effect of plant diversity on soil C and N storage. However, these experiments all included legumes, which constitute an important N input through N<sub>2</sub> fixation. Indeed, the results of these experiments suggest that N<sub>2</sub> fixation by legumes is a major driver of soil C and N storage. We studied if plant diversity affects soil C and N storage in the absence of legumes. In an 11-year grassland biodiversity experiment without legumes, we measured soil C and N storage. We further determined aboveground biomass productivity, standing root biomass, soil organic matter decomposition and N mineralization rates to understand the mechanisms underlying the change in soil C and N storage in relation to plant diversity and their feedbacks to plant productivity. We found that soil C and N storage increased by 18 and 16% in eight-species mixtures compared to the average of monocultures of the same species, respectively. Increased soil C and N storage were mainly driven by increased C input and N retention, resulting from enhanced plant productivity, which surpassed enhanced C loss from decomposition. Importantly, higher soil C and N storage were associated with enhanced soil N mineralization rates, which can explain the strengthening of the positive diversity-productivity relationship observed in the last years of the experiment. We demonstrated that also in the absence of legumes plant species richness promotes soil C and N storage via increased plant productivity. In turn, enhanced soil C and N storage showed a positive feedback to plant productivity via enhanced N mineralization, which could further accelerate soil C and N storage in the long term.

## Introduction

Understanding the relationships between biodiversity and ecosystem functioning is important for sustainable management of ecosystems. Most biodiversity studies have focused on primary production effects of plant biodiversity. The general picture emerging from these studies is that plant productivity increases with plant species richness (Cardinale et al., 2012). This pattern is attributed to facilitation and/or complementary resource use among species (Hooper et al., 2005) or a selection effect due to a higher chance of including a highly productive species in mixtures (Huston, 1997).

In the face of increasing atmospheric CO<sub>2</sub> concentrations and related climate change, accumulation of soil organic carbon (SOC) in terrestrial ecosystems is becoming another important ecosystem function, because in this process atmospheric carbon (C) is sequestered in soil. Only a few grassland studies have investigated the links between plant diversity and SOC dynamics. This may be related to the fact that accumulation of C is a slow process, and few biodiversity experiments are of sufficient duration to explore soil C dynamics and underlying mechanisms (but see De Deyn et al., 2009). These studies show positive effects of plant diversity on plant productivity and soil C sequestration (the Cedar Creek biodiversity experiment (Fornara and Tilman, 2008) and the Jena experiment (Steinbeiss et al., 2008)). Greater soil C accumulation in diverse species mixtures was mainly ascribed to increased soil C input from higher root biomass production. They also found increased soil fertility indicated by enhanced soil nitrogen (N) storage (Fornara and Tilman, 2008) and N mineralization (Dybzinski et al., 2008; Oelmann et al., 2011). However, both studies also showed strong effects of one particular functional group of plants, the legumes. In Cedar Creek, legumes have been identified as a key driver of primary productivity, C sequestration, N accumulation and mineralization (Hille Ris Lambers et al., 2004; Fornara and Tilman, 2008; Fornara and Tilman, 2009) and in the Jena experiment, legumes have also been shown to promote plant productivity (Marquard et al., 2009) and N availability (Oelmann et al., 2011). Legumes live in a symbiosis with Rhizobium bacteria, which fix atmospheric N and allocate it to the plant in exchange for

carbohydrates. Thus, the presence of legumes can strongly enhance the input of N into the ecosystem. As most grassland ecosystems are N-limited, including legumes will increase plant productivity, which in turn will lead to increased C sequestration in soil (Jones and Donnelly, 2004; Fornara et al., 2013). This is confirmed in a recent 2-year experiment, in which soil C and N pools were enhanced by the presence of two legume species, but not by species richness or functional group richness (De Deyn et al., 2009). Thus, although the contribution of legumes is an important mechanism, it remains to be established if plant diversity in the absence of legumes can enhance soil C and N storage. Recent studies have shown that species richness increased both aboveground and belowground biomass production in plant communities without legumes (Van Ruijven and Berendse, 2005; Mommer et al., 2010). We hypothesize that plant species richness promotes soil C and N storage in the absence of legumes, too, through enhanced biomass production.

Several studies have shown that diversity-productivity relationships strengthen with time (Cardinale et al., 2007; Van Ruijven and Berendse, 2009; Allan et al., 2011; Reich et al., 2012). It is still an open question which mechanism causes this. Potential explanations include increased functional turnover in species (Allan et al., 2011) and reduced negative impacts of soil biota at high diversity (Maron et al., 2011; Schnitzer et al., 2011; De Kroon et al., 2012). Here we test the hypothesis that the strengthening of the diversity-productivity relationship with time is due to positive ecosystem feedbacks, associated with C and N cycling (see also (Reich et al., 2012). It is well established that SOC increases with enhanced plant productivity (Jones and Donnelly, 2004), which in turn can positively feedback to plant productivity via enhanced decomposition and N mineralization. If diverse plots indeed store more C and/or retain more N than monoculture plots, and if the extra N subsequently becomes available for plant growth through N mineralization during the process of organic matter decomposition, then this would strengthen the diversity-productivity relationship in later years.

Here, we use an 11-year biodiversity experiment without legumes, which has shown a positive relationship between plant species richness and productivity (Van Ruijven and Berendse, 2009). First, we test whether plant species richness promotes soil C and N storage. Then, we test the effects of plant species richness on aboveground productivity, standing root biomass, SOC decomposition and soil N mineralization, and assess the potential for a positive feedback of SOC increase to plant productivity via soil N mineralization.

## Materials and methods

### **Experimental setup**

The Wageningen biodiversity experiment has been described in detail previously (Van Ruijven and Berendse, 2003) and therefore only a brief description is given. The experiment was established on a former arable field in Wageningen, the Netherlands, in 2000 and consisted of 102 plots of 1 m<sup>2</sup>. In each plot, the original topsoil up till 45 cm depth was removed and replaced by a mixture of black soil and pure sand (1:3). The species pool consisted of four grass species (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., and *Holcus lanatus* L.) and four forbs (*Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., and *Rumex acetosa* L.). The experimental plots were distributed over six replicated blocks. Each block contained seventeen treatments: all eight monocultures, four mixtures of two species, four mixtures of four species and one eight-species mixture. Species composition of two-species and four-species mixtures was selected randomly from the species pool, with the restriction that a particular combination could not be selected twice. In each plot, 144 seedlings were planted following a substitutive design.

### **Plant biomass**

Each year from 2000 to 2010, plants were clipped at 2.5 cm above the soil surface in late August, sorted to species and dried at 70 °C for at least 48 h before weighing. To avoid edge effects, only plants in the centre (60 cm × 60 cm) were used. Belowground standing

biomass was determined once in June, 2010. In a  $30 \times 30$  cm subplot within each plot, six soil cores (diameter 3 cm) were taken to a depth of 50 cm, divided into five layers: 0-5, 5-10, 10-20, 20-35 and 35-50 cm. Roots were collected by rinsing the samples using sieves (mesh size 1 mm). Fresh weight of the roots was determined after standardized drying of the samples between paper tissues (Mommer et al. 2010).

### ***Soil organic carbon and nitrogen storage***

Stratified soil sampling was performed using cylinders ( $100 \text{ cm}^3$ ) in April 2011 to assess soil C and N. Two samples, at 0-7.5 cm and 7.5-15 cm depths, respectively, were taken in all 102 plots. Intact ring samples were first weighted and then oven-dried at  $105^\circ\text{C}$  for 48 h before weighting again to determine dry bulk density and soil moisture content. Soil samples were sieved (2 mm mesh) to remove roots, mixed and ground in a ball mill. A subsample of 100 mg was analysed for soil C and N concentrations on Elementar Vario EL C/N analyser (Hanau, Germany). Soil C and N storage per depth ( $\text{g m}^{-2}$ ) was calculated by multiplying soil C and N concentrations by soil bulk density by soil depth. Summing up the soil C and N storage of the two depths resulted in the soil C and N storage up to 15 cm depth.

### ***Soil organic carbon decomposition and soil nitrogen mineralization***

Approximately 200 g field moist soil was sampled up to 15 cm depth in April 2011. Due to logistic constraints, this sampling was restricted to the first four (out of six) blocks, i.e.  $N = 68$ . Soil samples were sieved (2 mm) to remove roots, macro-fauna and debris. Subsamples (100 g dw equivalent) were incubated in bottles closed with a cotton plug at  $20^\circ\text{C}$  in the dark for 20 weeks. The subsamples were kept at 60 % of water holding capacity by supplying deionized water each 2 weeks. Efflux of  $\text{CO}_2$  was measured four times: at 2, 4, 8 and 20 weeks after filling the bottles. Prior to these measurements, the bottles were flushed with compressed air for 15 min and closed with an air-tight lid and then incubated in the dark for 4 h at  $20^\circ\text{C}$ . The increased  $\text{CO}_2$  concentration after this 4 h incubation was measured using a photo-acoustic gas monitor (INNOVA 1412, AirTech Instruments, Ballerup, Denmark). These numbers were extrapolated to  $\text{CO}_2$  production



per day (24 h) by multiplying them by six. The derivative of a first order exponential decay model as below was fitted to the daily CO<sub>2</sub> production rates using non-linear regression (SPSS 20.0).

$$C_{rate}(t) = k_l C_l \exp(-k_l t)$$

where  $C_{rate}(t)$  is daily CO<sub>2</sub> production rate (mg CO<sub>2</sub>-C kg<sup>-1</sup> soil day<sup>-1</sup>) present at time  $t$ ,  $C_l$  is the labile C pool (mg C kg soil<sup>-1</sup>) and  $k_l$  is the relative decomposition rate of a labile C pool. Ordinary sums of squares were used as goodness of fit criterion. Once the model described the data satisfactorily, we used the calibrated parameters  $k_l$  and  $C_l$  to estimate cumulative CO<sub>2</sub> production (mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) over the 20 weeks of incubation. We used this cumulative CO<sub>2</sub> production to represent SOC decomposition.

Two 20-g subsamples of soil were used to measure potential net N mineralization. One subsample was extracted immediately with 50 mL of 1 M KCl for mineral N analysis. The other subsample was adjusted to 65 % of water holding capacity, covered with polyethylene film and incubated in the dark at 29 °C for 6 weeks. Soil moisture content was kept constant throughout the incubation period. After incubation, soil samples were extracted again for mineral N. The KCl extracts were filtered using Whatman filter paper No. 2 and the filtered extracts were analysed for their concentration of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N using a continuous flow N analyser (Skalar, SAN plus, the Netherlands). The potential soil net N mineralization rate (µg kg<sup>-1</sup> day<sup>-1</sup>) was calculated by subtracting pre-incubation soil mineral N from post-incubation soil inorganic N concentrations and dividing it by the incubation period of 42 days.

## **Statistics**

In order to compare the relationships between species richness and different ecosystem functions, and between species richness and aboveground biomass in different years, we fitted power functions (Cardinale et al., 2011; Reich et al., 2012).

Aboveground productivity over time was analysed using a mixed model, in which plot biomass in different years was treated as repeated observations, with block as random

factor, year as a fixed factor and  $\log_2$  of species richness as a covariate. For comparison with the other ecosystem functions, we also determined the relationship between species richness and the average aboveground biomass of each plot over the 11 years of the experiment (as a measure of mean plant productivity), using a mixed model with block as a random factor.

The relationship between species richness and root biomass was analysed using a mixed model, in which root biomass at different soil depths within a plot was treated as repeated observations, with block as random factor, depth as a fixed factor and  $\log_2$  of species richness as a covariate. Similar to aboveground biomass, a power function was fitted.

To analyse the effect of species richness on soil C and N storage and the soil C:N ratio at different soil depths, a mixed model was used in which the values at different depths within plots were considered repeated observations. In this analysis, block was included as a random factor, depth was included as a fixed factor and  $\log_2$  of species richness as a covariate. In case of a significant interaction, a mixed model was used to determine the relationship between species richness at each soil depth.

Mixed models were also used to analyse the relationships between species richness and SOC decomposition and N mineralization, with block as random factor and  $\log_2$  of species richness as a covariate. To determine if differences in productivity could explain the positive effects of species richness on soil C and N storage, SOC decomposition and N mineralization, mixed models were used as described above, but in this case aboveground biomass production and standing root biomass was fitted separately before species richness using type I sums of squares.

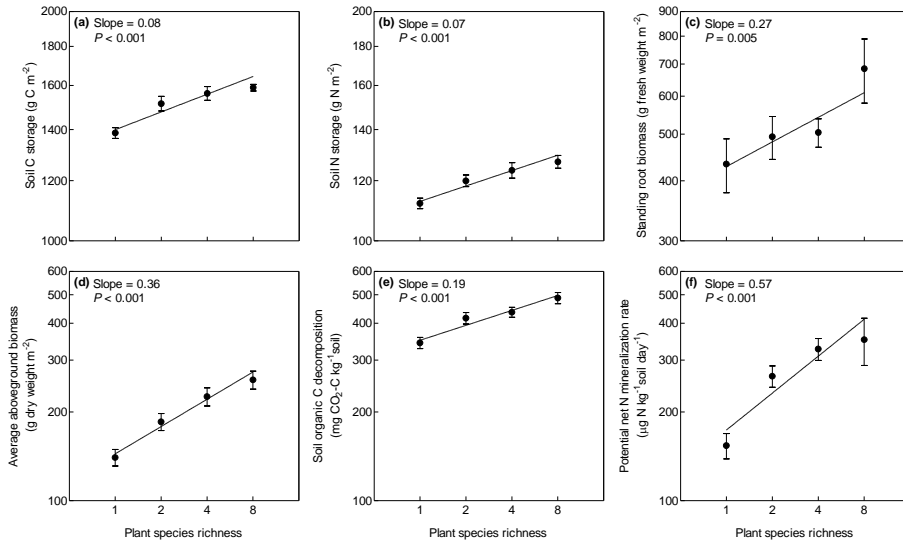
Ideally, we would have liked to partition a species richness effect on ecosystem functions into a complementarity effect and a selection effect (Loreau and Hector, 2001). However, this was not possible because the data from each component species in mixtures were not available for most ecosystem functions. Thus, we followed an alternative three-step

approach to identify the effects of a particular species on ecosystem functions. First, we tested for differences among species in monocultures. If significant, we tested if the ecosystem function in question was enhanced by the presence of the species with the highest value for a particular ecosystem function in two- and four-species mixtures (eight-species mixtures contain each species and therefore could not be included in this analysis). Finally, we tested for significant relationships with species richness in the plots without (i.e. from monocultures to four-species mixtures) and in plots with that particular species. Specifically, we focused on root biomass at 0-20 cm depth in this analysis to allow comparison with the soil C and N storage data (0-15 cm depth). The statistical models used in this procedure were similar to the ones described above for each ecosystem function. All analyses were performed using SPSS 20.0 (IBM).

## Results

### ***Soil organic carbon and nitrogen storage***

Soil C storage increased with species richness ( $F = 28.5$ ;  $P < 0.001$ ). The slope of the relationship between species richness and soil C storage was  $0.08 \pm 0.02$  (Fig. 2.1a), which represents an average increase of 6% in soil C storage (0-15 cm) with each doubling of species richness. More C was stored in the top layer (0-7.5 cm) than in the second soil layer (7.5-15 cm) ( $F = 505.4$ ;  $P < 0.001$ ), but soil depth did not affect the relationship between species richness and soil C storage (species richness  $\times$  depth:  $F = 0.5$ ;  $P = 0.48$ ). In monocultures, soil C storage varied among species ( $F = 2.3$ ;  $P = 0.04$ ). It was greater in monocultures of *C. jacea* and *L. vulgare* than in most other species (Fig. S2.1a). In two- and four-species mixtures, *C. jacea* enhanced C storage ( $F = 7.9$ ;  $P < 0.01$ ), but the presence of *L. vulgare* had no effect ( $F = 0.3$ ;  $P = 0.60$ ). These effects were independent of soil depth. When plots with and without *C. jacea* were analysed separately, C storage increased significantly with species richness in plots without ( $F = 11.7$ ;  $P < 0.01$ ), but not in plots with this species ( $F = 0.9$ ;  $P = 0.36$ ).



**Figure 2.1** Relationships between species richness and a) soil C storage (0-15 cm), b) soil N storage (0-15 cm), c) standing root biomass (up to 50 cm), d) average aboveground biomass (2000-2010), e) soil organic C decomposition (measured as  $\text{CO}_2$  production), and f) potential net N mineralization rate. Lines show the fitted  $\log_2$ - $\log_2$  relationships. Data points show means  $\pm$  SE.

Soil N storage also increased with species richness ( $F = 20.5$ ;  $P < 0.001$ ). The slope of this relationship ( $0.07 \pm 0.01$ ) is similar to that of the relationship between soil C storage and species richness (Fig. 2.1b). The N storage was greater in the top layer (0-7.5 cm) than in the second soil layer (7.5-15 cm) ( $F = 476.4$ ;  $P < 0.001$ ), but soil depth did not affect the relationship between species richness and soil N storage (species richness  $\times$  depth:  $F = 0.1$ ;  $P = 0.77$ ). In monocultures, soil N storage did not differ among species ( $F = 1.7$ ;  $P = 0.15$ ; Fig. S2.1b).

The soil C:N ratio increased with species richness ( $F = 4.4$ ;  $P < 0.05$ ) and not affected by soil depth ( $F = 0.5$ ;  $P = 0.49$ ). The species richness effect on soil C:N ratio was minor, though (slope =  $0.02 \pm 0.01$ ). The soil C:N ratio did not vary among species grown in monoculture ( $F = 1.3$ ;  $P = 0.26$ ).

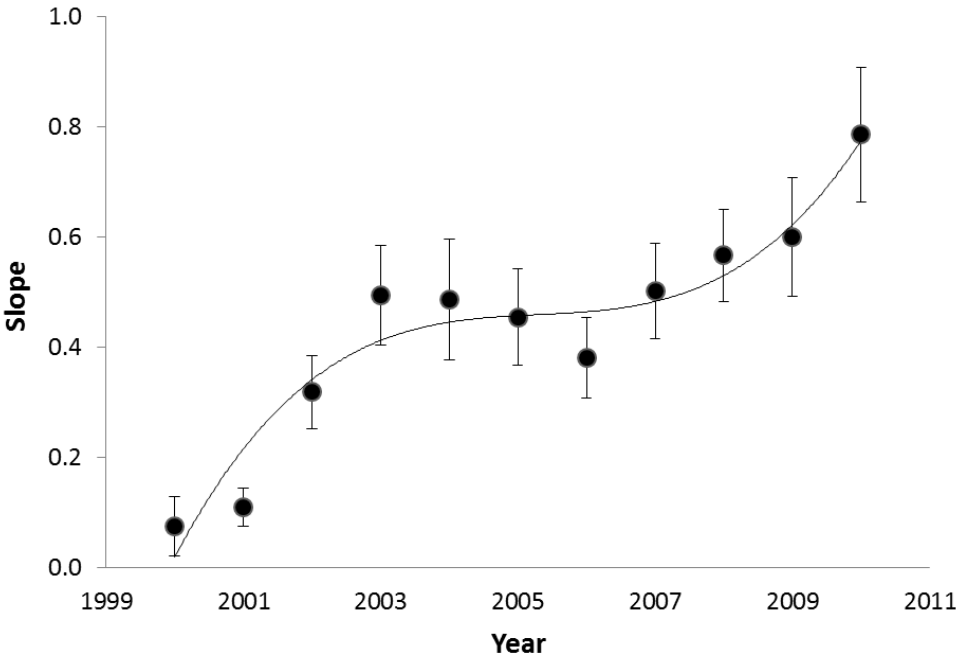
### **Root biomass**

Root biomass increased with species richness ( $F = 13.6$ ;  $P < 0.001$ ) from  $433 \text{ g m}^{-2}$  on average in monoculture to  $685 \text{ g m}^{-2}$  on average in the mixture of the eight plant species. Root biomass strongly differed among soil layers ( $F = 64.4$ ;  $P < 0.001$ ). The top 10 cm contained more than 73% of the root biomass, whereas the deepest layer (35-50 cm) contained only 6%. Soil depth only marginally affected the relationship between species richness and standing root biomass (species richness  $\times$  depth:  $F = 2.3$ ;  $P = 0.06$ ). The slope of the power function between total root biomass (0-50 cm) and species richness ( $0.27 \pm 0.09$ ) was larger than for soil C storage (Fig. 2.1c) and corresponds to a 20% increase in root biomass with each doubling of species richness.

Within monocultures, species richness affected root biomass significantly ( $F = 4.1$ ;  $P < 0.01$ ): root biomass of *F. rubra* was greater than that of all other species (Fig. S2.1c), although the differences between *F. rubra* and *C. jacea* ( $P = 0.35$ ) and *F. rubra* and *A. capillaris* ( $P = 0.09$ ) were not significant. In two- and four-species mixtures, however, the presence of *F. rubra* did not affect root biomass ( $F = 1.7$ ;  $P = 0.19$ ). The presence of *C. jacea* increased root biomass in these mixtures, but this effect depended on soil depth (presence  $\times$  depth:  $F = 6.2$ ;  $P < 0.01$ ) and only occurred in the deeper soil layer (10-20 cm). In plots without *C. jacea*, root biomass showed a marginally significant increase with species richness ( $F = 6.2$ ;  $P = 0.06$ ), independent of soil depth (species richness  $\times$  depth:  $F = 1.4$ ;  $P = 0.26$ ). In plots with *C. jacea*, however, the effect of species richness depended on soil depth (species richness  $\times$  depth:  $F = 4.2$ ;  $P < 0.01$ ) and was significant (and positive) only in the top layer (0-5 cm). The presence of *A. capillaris* did not affect root biomass in two- and four-species ( $F = 0.2$ ;  $P = 0.63$ ).

### **Above-ground plant productivity**

Aboveground productivity increased with species richness ( $F = 47.9$ ;  $P < 0.001$ ), but the strength of the effect depended on duration of the experiment (species richness  $\times$  time:  $F = 6.8$ ;  $P < 0.001$ ). When analysed for each individual year, the relationship between species richness and aboveground productivity was significant in each year except for the



**Figure 2.2** The slopes of the power function between aboveground productivity and species richness in relation to experimental years. This relationship was described well by a cubic function (shown by the line). Data points show the mean slope  $\pm$  SE.

first. The relationship between time and the slope of the power function between species richness and aboveground productivity could best be described by a cubic function (Fig. 2.2;  $F = 28.4$ ;  $P < 0.001$ ;  $r^2 = 0.924$ ). In the first 3 years, this slope increased with time. In the following 4 years it levelled off (as we reported previously, Van Ruijven and Berendse, 2009). However, in the last 4 years, the slope increased again. We tested the latter by fitting the linear and quadratic term before the cubic term in a GLM with sequential sums of squares. The cubic term remained significant ( $F = 10.1$ ;  $P < 0.05$ ). For comparison with root biomass and C and N storage, we also included the average aboveground biomass over 2000 – 2010. It showed a significant positive relationship with species richness ( $F = 41.5$ ;  $P < 0.001$ ) with a slope of  $0.36 \pm 0.06$  (Fig. 2.1d), which is comparable to that of root biomass (slope =  $0.27 \pm 0.09$ ).

When the monocultures were analysed separately, aboveground biomass differed among species ( $F = 17.5$ ;  $P < 0.001$ ) and years ( $F = 114.6$ ;  $P < 0.001$ ), and the differences among species were time-dependent (species  $\times$  time:  $F = 10.7$ ;  $P < 0.001$ ). In most years (except for 2000, 2001 and 2007), *C. jacea* was the most productive species (Fig. S2.1d). In two- and four-species mixtures, the presence of this species enhanced aboveground biomass ( $F = 60.2$ ;  $P < 0.001$ ), although the size of this effect differed among years (presence  $\times$  year:  $F = 4.7$ ;  $P < 0.001$ ). When analysing all plots with *C. jacea* separately, no general relationship between aboveground biomass and species richness was observed ( $F = 0.2$ ;  $P = 0.63$ ). Instead, the relationship differed among years (species richness  $\times$  year:  $F = 3.4$ ;  $P < 0.01$ ), with the slope ranging from zero in the first 2 years, decreasing to negative from 2002 until 2006 (significant in 2005). From 2006 onwards, the slope increased again and was positive from 2007 to 2010. In the last year, this slope was significantly different from zero ( $F = 5.5$ ;  $P < 0.05$ ). In contrast, the patterns found in plots without *C. jacea* were similar to the overall patterns for aboveground biomass. There was an overall positive effect of species richness on aboveground biomass ( $F = 26.8$ ;  $P < 0.001$ ). The slope differed among years (species richness  $\times$  year:  $F = 4.0$ ;  $P < 0.001$ ), gradually increasing from 0.07 in 2000 to 0.73 in 2010.

### **Soil organic carbon decomposition**

The first order exponential decay model described CO<sub>2</sub> efflux well, with an average  $r^2$  of  $0.94 \pm 0.03$ . The estimated cumulative CO<sub>2</sub> production (mg CO<sub>2</sub>-C kg<sup>-1</sup> soil) over the 20 weeks of incubation increased with species richness ( $F = 36.8$ ;  $P < 0.001$ ). The slope of the relationship ( $0.19 \pm 0.03$ ) was smaller than for root biomass, but greater than for soil C storage (Fig 2.2e). To correct for differences in soil C content, we repeated the analysis but fitted soil C content as a covariate before species richness, using type I sums of squares (Hector et al., 2010). The cumulative CO<sub>2</sub> production showed a strong increase with an increasing soil C storage ( $F = 76.5$ ;  $P < 0.001$ ), but the effect of species richness on CO<sub>2</sub> production remained significant ( $F = 4.7$ ;  $P < 0.05$ ), with a slope of  $0.07 \pm 0.03$ . The cumulative CO<sub>2</sub> production did not differ between species in monoculture ( $F = 1.5$ ;  $P = 0.23$ ; Fig. S2.1e).

**Soil nitrogen mineralization**

Plant species richness had a strong positive effect on potential soil net N mineralization ( $F = 37.2$ ;  $P < 0.001$ ): soil N mineralization increased on average by 48% with each doubling of species richness (Fig. 2.1f). The strong relationship occurred even when we adjusted the rate of N mineralization for differences in soil C content (slope =  $0.56 \pm 0.11$ ). Potential net N mineralization rates did not differ among monocultures ( $F = 0.7$ ;  $P = 0.65$ ; Fig. S2.1f).

**Plant species richness or plant productivity**

In general, average aboveground productivity was a better predictor for soil C and N storage as well as SOC decomposition, soil N mineralization and soil C/N ratio than standing root biomass (Table 2.1). When aboveground productivity was fitted before species richness, the latter remained significant, but the slope was reduced to a large extent (Table 2.1). When root biomass was fitted before species richness, the latter remained highly significant. Moreover, the relationship with root biomass was often relatively weak (Table 2.1).

**Table 2.1** Slopes of the power relationships between species richness and different soil properties. The first model (1) refers to the mixed model used in the main analysis in which only species richness was fitted. In the other two models (2, 3), aboveground productivity (shoot production) and standing root biomass, respectively, were fitted before species richness using type I sums of squares.

Model	Soil C storage	Soil N storage	SOC decomposition	Soil N mineralization	Soil C:N ratio
1) Species richness	0.08***	0.07***	0.19***	0.57***	0.02*
2) Shoot production	0.11***	0.08***	0.17***	0.38*	0.02**
Species richness	0.04*	0.04*	0.12**	0.32***	0.00 <sup>NS</sup>
3) Standing root biomass	0.04**	0.05**	.13***	0.16 <sup>NS</sup>	0.01 <sup>NS</sup>
Species richness	0.07***	0.05**	.14***	0.39***	0.01 <sup>NS</sup>



## Discussion

Our results show that plant species richness enhanced soil C and N storage, thereby extending the results of previous studies (Fornara and Tilman, 2008; Steinbeiss et al., 2008) to grasslands without legumes. Hence, we, for the first time, provided evidence that additional input of N by biological N<sub>2</sub> fixation is not a prerequisite for enhanced soil C and N storage. Greater soil C and N storage at higher species richness was mainly attributed to increased soil C input and N retention, rather than N input, with enhanced plant productivity.

It is important to note that the presence of the forb *C. jacea* enhanced plant biomass and soil C storage. This is consistent with results from the Jena experiment, which showed that the presence of tall forb species (including *C. jacea*) reduced soil C losses (Steinbeiss et al., 2008). However, it is clear from our results that the presence of this species is not the only driver of species richness effects: species richness also enhanced plant biomass and soil C storage in plots without *C. jacea*. Moreover, three other processes (N storage, SOC decomposition and N mineralization), which increased with species richness, were not affected by the presence of *C. jacea*. Therefore, we conclude that the positive effects of plant species richness in our experiment are not solely due to a single species (see also Van Ruijven and Berendse, 2005, 2009).

We hypothesized that in the long run, enhanced soil C and N storage would lead to a positive feedback to plant productivity. This is supported by the increase in net potential N mineralization rate with increasing species richness we observed. This increased N mineralization is likely to fuel a further increase of primary productivity through increased N availability. This may explain the strengthening of the positive biodiversity-productivity relationship we observed in the later years (2008-2010). These findings are similar to those of two other long-term biodiversity experiments in Cedar Creek including legumes, which also showed positive effects of plant species richness on N mineralization (Dybziński et al., 2008; Mueller et al., 2013) and increasingly positive effects on plant productivity over

time (Reich et al., 2012). In contrast to what is generally assumed, our findings imply that these feedbacks are not necessarily dependent upon the presence of legumes.

The positive effect of species richness on soil C storage can be caused by increased soil C input (via enhanced production) and/or decreased C losses due to decreased decomposition rates. In this study, the consistently positive effects of species richness on aboveground productivity and the positive relationship between average annual aboveground productivity and soil C storage suggest that plant species richness promotes C inputs into the soil. As aboveground biomass was to a large extent removed from the plots each year, the main input of organic C and N was as root biomass and root exudates. Root biomass also increased with species richness in our experiment. However, compared to aboveground productivity, root biomass was a poor predictor of soil C and N storage. This can be explained by the fact that aboveground productivity, averaged over the whole 11 years of the experiment, is a better proxy for plant input into the soil than standing root biomass measured only once (in 2010).

In contrast to what has been suggested in other studies (Fornara et al., 2009; De Deyn et al., 2012) we found that SOC decomposition (measured as CO<sub>2</sub> production) increased with species richness. This effect persisted after accounting for differences in the total amount of SOC, indicating that species richness increased the relative decomposition rates of SOC. Two potential mechanisms may explain this acceleration. First, increased plant productivity with higher plant species richness and consequent higher organic matter inputs into soil may have led to a faster rejuvenation of the SOC pool. This may have reduced the recalcitrance of SOC (Dijkstra et al., 2005). Second, plant species richness can supply a greater diversity of organic compounds and stimulate more diverse microbial decomposer communities (Stephan et al., 2000) and a higher diversity of extracellular enzyme production (Fontaine et al., 2003), thereby enhance the decomposition rate of SOC (Meier and Bowman, 2008; Meier and Bowman, 2010).

Increased soil C storage with increasing species richness in our experiment is the result of increased C inputs through biomass production and enhanced SOC decomposition. Clearly, the balance between the two is tipping towards the first in our experiment. This is supported by the comparison of slopes of the relationships between species richness and biomass production, and species richness and SOC decomposition, the latter being smaller (Fig. 2.1).

The increase in soil N storage in more diverse plots was very similar to that of C. This seems counterintuitive because, in contrast to experiments including legumes, the only N input in this system is atmospheric N deposition, which ranges from approximately 1 - 2.5 g m<sup>-2</sup> year<sup>-1</sup> in this part of the Netherlands (Limpens et al., 2004). This input is equal for each treatment. It is complicated further by the larger N removal from the more diverse plots with biomass harvests (Van Ruijven and Berendse, 2005). Based on N concentrations in aboveground biomass and the amount of biomass harvested in 2002 and 2008, the total amount of N removed (g m<sup>-2</sup>) in monoculture was  $1.5 \pm 0.1$  and  $1.1 \pm 0.1$ , whereas in eight-species mixtures, it was  $2.2 \pm 0.7$  and  $2.3 \pm 0.3$ , respectively. This suggests that in high species-richness plots, annual N removal was balanced by N deposition, whereas N deposition exceeded N removal in monocultures.

Consequently, increased N storage in treatments with higher species richness can only be explained by a substantial decrease in N losses. Indeed, several grassland studies have shown that species richness decreases N leaching (Hooper et al., 2005; Dijkstra et al., 2007; Oelmann et al., 2007; De Deyn et al., 2009). There are several potential explanations: First, more N can be reallocated from deeper soil layers to the top soil (where most uptake will take place) due to larger root biomass throughout the soil profile in more diverse plots. Second, N leaching can be reduced due to more variation in phenology across species in more diverse plots, resulting in N uptake during a larger part of the growing season (McKane et al., 1990). This variation in phenology could also lead to a more efficient N recycling within the growing season because N mineralisation of decomposing roots from early species may provide later species with N that would

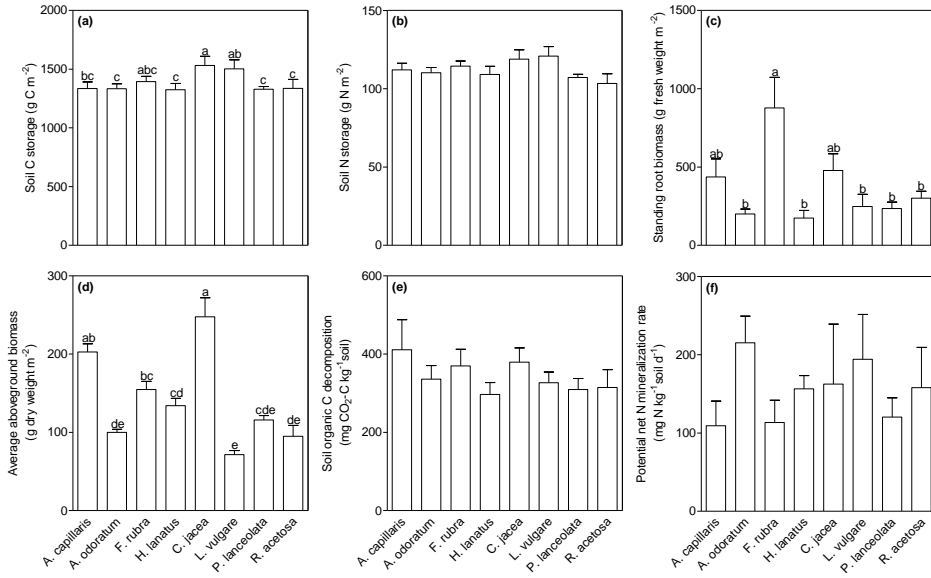
otherwise have been lost through leaching. The observed enhanced potential mineralisation in more diverse plots supports this explanation.

In conclusion, we have shown that plant species richness promotes soil C and N storage mainly through enhanced plant productivity, despite accelerated SOC decomposition. Increased soil C and N storage appears to show a positive feedback to plant productivity via enhanced net N mineralization rates in the last years, in which the positive relationship between plant species richness and productivity became stronger. Importantly, these effects occurred in the absence of N<sub>2</sub> fixation by legumes, suggesting that similar results obtained in experiments with legumes were at least not solely due to the inclusion of legumes. These findings suggest that more diverse ecosystems can increase the potential for C sequestration in terrestrial ecosystems, at least in the term of a decade.

## Acknowledgements

We thank Maurits Gleichman, Frans Möller, Jaap Nelemans, Junqi Zhu, Karst Brolsma and Jan van Walsem for field and laboratory assistance. The Wageningen biodiversity experiment was supported by a grant from the Dutch Organization for Scientific research (NWO) within the stimulation program Biodiversity. G.D.D. and L.M. acknowledge the NWO-ALW VIDI and VENI grant schemes, respectively for financial support.

## Supporting information



**Figure S2.1** Characteristics for the eight monocultures: a) soil C storage (0-15 cm), b) soil N storage (0-15 cm), c) standing root biomass (up to 20 cm), d) average aboveground biomass (2010-2010), e) soil organic C decomposition, and f) potential net N mineralization rate. Data are means  $\pm$  SE. Different letters denote significant ( $P < 0.05$ ) differences between monocultures based on Tukey post hoc tests.



## Chapter 3

### **Plant species richness accelerates decomposition of root litter through a soil-mediated effect**

Wenfeng Cong, Jasper van Ruijven, Wopke van der Werf,  
Liesje Mommer and Ellis Hoffland



### **Abstract**

Enhanced belowground biomass production in species diverse plant communities may enhance carbon input in soil, but accumulation of C in soil depends critically on the effect of diversity on the rate of breakdown of organic matter. The rate of decomposition could be affected by litter diversity or by changes in abiotic or biotic attributes of the soil, associated with plant species richness. Previous studies examined the litter diversity and soil mediated effects separately, and do therefore not elucidate the relative importance of the litter diversity and soil environment effects, and their potential interaction. Here we separated the effects of litter mixing from those mediated by alteration of the soil using a laboratory incubation experiment. The soil-mediated effect was further examined using a recalcitrant standard substrate (compost). We found that the mixture of root litter decomposed as expected from single litters, indicating absence of a litter mixing effect. The rate of decomposition of root litter (single litter or mixture) was greater in soils from high diversity plots than in soils from plant species monocultures, indicating that plant species richness enhanced root litter decomposition through a soil-mediated effect. But this effect was not observed for compost decomposition. These results suggest that differences in the soil decomposition environment between species poor and species rich plots affect the rate of decomposition of labile organic matter. We demonstrated that plant species richness enhances root decomposition via changes in soil ecosystem but not via litter mixing. Enhanced root decomposition may weaken the impacts of species richness on soil C sequestration potential.



## Introduction

Studies investigating the relationship between biodiversity and ecosystem functioning focus on the effect of plant diversity on productivity. Generally, plant productivity increases with plant species richness (Cardinale et al., 2012). This increase is attributed to causal mechanisms such as complementary resource use or facilitation among species (Hooper et al., 2005) or generated by chance through a selection effect (Huston, 1997). Enhanced plant productivity, resulting in greater litter input, could result in more soil carbon (C) sequestration, provided that decomposition of organic matter is not stimulated (Catovsky et al., 2002). Indeed, two grassland biodiversity experiments have recently demonstrated that plant species richness promotes C accumulation in soil through enhanced root biomass production, indicating that effects of plant diversity on rate of decomposition are absent or modest (Fornara and Tilman, 2008; Steinbeiss et al., 2008). In a previous study, we found that plant productivity increased by 84% in eight-species mixtures compared to the average of monocultures of the same species, while soil C storage after 11 years increased less, by 18% (Cong et al., submitted). This discrepancy could potentially be explained by enhanced decomposition of soil organic matter. In this study, we examine whether and how species richness affects the rate of decomposition of root litter.

Plant species richness can potentially affect litter decomposition through changes in litter composition (Wardle and Lavelle, 1997). Studies using a gradient of litter diversity have shown no consistent effect of litter diversity on litter decomposition (Hattenschwiler et al., 2005; Mikola et al., 2002; Wardle et al., 1997). Non-additive litter mixing effects prevail in these studies. In other words, mixing litters can accelerate or inhibit decomposition compared to the expected decomposition of the single litters (Gartner and Cardon, 2004; Hattenschwiler et al., 2005). This phenomenon is commonly observed when the components of litter mixture vary in resource quality (Wardle et al., 1997), suggesting that mixing litters from different functional groups of plant species could cause a non-addition effect. For example, a high-N legume or forb litter can be more readily decomposed by

soil microorganisms and the N released from the high-N litter may then facilitate the decomposition of a low-N grass litter (Harguindeguy et al., 2008; Vos et al., 2013; Wardle et al., 1997). Similarly, when one of the litter components contains inhibitory compounds (e.g. polyphenols), this will inhibit microbial growth and activity of the whole community, consequently impeding decomposition of both litters (Schimel et al., 1998). Most of the current studies focus on leaf litter decomposition. Little is known about the effect of litter mixing on the decomposition of root litter.

Another potential mechanism through which species richness could affect organic matter decomposition is through alteration of the decomposition microenvironment, i.e. through changes in abiotic and biotic attributes of the soil (hereafter referred to “soil ecosystem effect”) (Hector et al., 2000). Based upon grassland biodiversity experiments, several studies showed that plant species richness enhances the decomposition of standard litter via a soil-mediated effect (i.e. changes in the decomposition microenvironment) (Hector et al. 2000; Knops, Wedin & Tilman 2001; Spehn et al., 2005; Vogel et al., 2013). But no effect of plant species richness on decomposition was found at the Swiss and German sites of the BIODDEPTH project (Spehn et al., 2000; Scherer-Lorenzen, 2008). While previous studies have examined the two potential pathways (i.e. litter mixing effect and soil ecosystem effect) independently, it is essential to disentangle the two pathways and compare their relative impacts on litter decomposition in one study and assess whether there are interactions.

Here, we attempt to disentangle and compare the effects of litter mixing and soil ecosystem on root litter decomposition. A factorial design experiment with soil and root litter as two factors was conducted in a factorial laboratory incubation experiment to unravel the two effects. Soil samples were taken in plots of monocultures, two-species and four-species mixtures from an 11-year grassland biodiversity experiment (Van Ruijven and Berendse, 2003). The study focuses on root litters from one grass species and one forb species.

## Materials and methods

### **Field sampling**

The Wageningen biodiversity experiment is described in detail in Van Ruijven and Berendse (2003). Thus, only a brief description is given here. The experiment was established on a former arable field in Wageningen, the Netherlands in early spring 2000 and consisted of 102 plots of 1 m<sup>2</sup>. The original topsoil up till 45 cm depth was removed and replaced by a mixture of black soil and pure sand (1:3). The species pool consisted of four C3 grasses (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., and *Holcus lanatus* L.) and four forbs (*Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., and *Rumex acetosa* L.). In each plot, 144 seedlings were planted following a substitutive design. The experimental plots were distributed over six blocks. Each block contained seventeen treatments: all eight monocultures, four mixtures of two species, four mixtures of four species and one eight-species mixture. Species composition of the two- and four-species mixtures were selected randomly without replacement from the species pool. This randomization was made independently for each block. Therefore, multi-species plots in different blocks consisted of a different random selection of species. In this study, we selected six treatments (Table 3.1) to examine the effect of species richness on root litter decomposition: two monocultures of *A. odoratum* ("Ao") and *C. jacea* ("Cj"), one mixture of the two species ("Ao+Cj") and three mixtures of four species: one containing *A. odoratum* plus three other species excluding *C. jacea* ("Ao+3"), another containing *C. jacea* plus three other species excluding *A. odoratum* ("Cj+3") and a third one including both *A. odoratum* and *C. jacea* plus two more species ("Ao+Cj+2"). The six soil treatments including *A. odoratum* and/or *C. jacea* were chosen based on three criteria: (1) the two species are from two different functional groups, representing a large possibility of the difference in root quality (2) the two species had sufficient fresh roots at the sampling time which enable us to conduct the following factorial design experiment and (3) the two species were dominant species in the last years of the experiment.

**Table 3.1** Treatments included in the factorial design experiment with soil and root litter (or compost) as two factors

Plant species richness	Soil <sup>a</sup> (n = 4)	Root litter			Compost	Control
		Ao	Cj	Ao/Cj <sup>b</sup>		
Monoculture	Ao	x	x	x	x	x
	Cj	x	x	x	x	x
Two-species	Ao+Cj <sup>c</sup>	x	x	x	x	x
Four-species	Ao+3	x	x	x	x	x
	Cj+3	x	x	x	x	x
	Ao+Cj+2	x	x	x	x	x

<sup>a</sup> Soil from six treatments: Ao, *A. odoratum*; Cj, *C. jacea*; Ao+Cj, *A. odoratum* grown with *C. jacea*; Ao+3, *A. odoratum* grown with other three species; Cj+3, *C. jacea* grown with three other species; Ao+Cj+2, *A. odoratum* grown with *C. jacea* and two other species.

<sup>b</sup> Ao/Cj refers to root mixture of *A. odoratum* and *C. jacea*.

<sup>c</sup> Four soil subsamples were taken from a single plot, while soil samples of the other five treatments were all taken from four different field replicates.

Soil samples (0-15 cm depth) were taken in each of the six treatments in four (out of six) blocks using a spade in April 2011. Given that the experiment contained only one single plot for the two-species mixture (Ao+Cj), we had 21 field samples in total (*i.e.* four blocks × five treatments + one “Ao+Cj”). Samples were sieved (2 mm), followed by removal of visible fresh plant material and macro-fauna and then stored at 4 °C until further processing. Fresh roots (< 2 mm diameter) of *A. odoratum* and *C. jacea* were collected from their monoculture plots. They were washed to remove soil particles, oven-dried at 70 °C for 48 h and cut into pieces of approximately 1 cm length. C and N content of root samples was determined prior to incubation by Elemental Vario EL C/N Analyser (Hanau, Germany). Lignin concentration of root samples was determined by the acid detergent lignin method (Van Soest, 1967).

### **Laboratory incubation experiment**

The laboratory experiment was set up as a two-way factorial design with “soil” (six levels; Table 3.1) and “root litter” (three levels: Ao, Cj and Ao/Cj mixture) as factors. Each combination was replicated four times with the soils from four field blocks, except in the

case of the “Ao+Cj” for which we had a soil sample from only one plot. In this case, four soil subsamples were taken from the single soil sample to substitute for the lack of true replicates at plot level. Within and between plot variances were compared to assess whether these pseudoreplicates could be used as substitutes for true replicates. A factorial design was chosen to separate effects of soil ecosystem from those of root litter mixing on root decomposition, and establish whether or not those effects interact.

In addition to the three root litters, compost (C/N ratio of 16) was added to the factorial design as a standard substrate and fourth level for the factor “root litter” (Table 3.1) to test the soil ecosystem effect on long-term decomposition. Compost is a recalcitrant material which decomposes much more slowly than fresh plant material (e.g. root litter). In total, we had 96 substrate-soil mixtures (six soils  $\times$  four substrates  $\times$  four replicates). Finally, controls were included, consisting of each of the six soils without added substrates.

A standard amount of 0.5 g oven-dried (70°C) substrate (root litter or compost) was added to moist soil (50 g on dry weight basis). The amount of substrate added to soil ensured sufficient amount of C for microbial respiration over the incubation period. The root mixture (Ao/Cj) contained equal amounts of roots of *A. odoratum* and *C. jacea*. Controls without substrate were incubated for each of the six soils to monitor the CO<sub>2</sub> production from soil organic matter. The mixtures of soil and substrates were kept in 250 mL-bottles that were closed with a gas-permeable cotton plug and incubated in the laboratory at 20 °C. Soil moisture content was kept at 60% of water holding capacity by supplying deionized water every 2 weeks. The production of CO<sub>2</sub> was measured at 1, 3, 7 and 15 weeks after filling the bottles. Prior to these measurements, the bottles were flushed with compressed air for 15 minutes and closed with an air-tight lid and then incubated in the dark at 20 °C for 4 h. The increase in CO<sub>2</sub> concentration after this 4 h incubation was measured using a photo-acoustic gas monitor (INNOVA 1412, AirTech Instruments, Ballerup, Denmark).

### **Calculations and statistical analysis**

Substrate (root litter or compost)-derived CO<sub>2</sub> production was calculated by subtraction of the CO<sub>2</sub> efflux from soil alone from the total CO<sub>2</sub> efflux produced by substrate-amended soil, assuming additivity.

To quantitatively compare species richness effects on decomposition of root litter or compost, we used linear interpolation between subsequent measurements to calculate the cumulative amount of C decomposed (*CCD*, g CO<sub>2</sub>-C kg<sup>-1</sup> soil; Muller-Stover et al., 2012). *CCD* was expressed as a percentage of C decomposed, taking into account the initial amount of C in the root sample:

$$CD (\%) = (CCD/C_0) * 100 \quad (\text{Eq. 1})$$

where  $C_0$  is the amount of C from the root sample at  $t = 0$  (g C kg<sup>-1</sup> soil). The expected *CD* (%) of the mixed root sample was calculated on the basis of the weighted root C from *A. odoratum* and *C. jacea* as follows:

$$CD_{exp}(\%) = CD_{Ao}(\%) \frac{C_{Ao}}{C_{Ao} + C_{Cj}} + CD_{Cj}(\%) \frac{C_{Cj}}{C_{Cj} + C_{Ao}} \quad (\text{Eq. 2})$$

where  $CD_{Ao}$  (%) and  $CD_{Cj}$  (%) are the percentage of C decomposed from the root sample of *A. odoratum* and *C. jacea*, respectively.  $C_{Ao}$  and  $C_{Cj}$  are initial C concentration (%) of root samples of *A. odoratum* and *C. jacea*, respectively. While the shares of the two species in the root sample were 50/50 on dry weight basis, the higher C content of *A. odoratum* roots resulted in a slightly higher contribution of this species to total C in the sample.

One-way analysis of variance was used to analyse the differences in initial chemical characteristics between roots of *A. odoratum* and *C. jacea*. The relationships between plant species richness and decomposition of root litter and compost were analysed using a general linear model (GLM) with block as a random factor, substrate (root litter or compost) as a fixed factor, and species richness as a covariate. The four soil subsamples from the single two-species mixture plot ( $A_o+C_j$ ) were assigned as the same block number in the model. In case of a significant interaction between substrate and species richness, a GLM was used to determine the relationship between species richness and decomposition for each substrate. A GLM was also used to test whether the rate of

**Table 3.2** Chemical characteristics of roots of two plant species (means  $\pm$  SEM,  $n = 4$ )

Species	% C	% N	C/N ratio	% Lignin	Lignin/N ratio
<i>A. odoratum</i>	39.0 $\pm$ 0.2 <sup>a</sup>	0.90 $\pm$ 0.03 <sup>a</sup>	43.5 $\pm$ 1.1 <sup>a</sup>	0.59 $\pm$ 0.26 <sup>b</sup>	0.66 $\pm$ 0.27 <sup>b</sup>
<i>C. jacea</i>	35.2 $\pm$ 0.5 <sup>b</sup>	0.78 $\pm$ 0.05 <sup>a</sup>	45.8 $\pm$ 2.5 <sup>a</sup>	4.36 $\pm$ 1.04 <sup>a</sup>	5.67 $\pm$ 1.33 <sup>a</sup>

Data in the same column with different lowercase letters are significantly different ( $P < 0.05$ ).

decomposition of mixed litter is significantly different from the weighted value of the single litters (*i.e.* litter mixing effect). To examine the effect of species composition (*i.e.* six soils) within monocultures and four-species mixtures, a mixed linear model was used with block as a random factor, species richness, species composition and root litter as three fixed factors. In the model, species composition was nested within species richness. All data met the ANOVA assumptions of normality and homogeneity of variance.

## Results

### **Initial chemical characteristics of root samples**

Roots of *A. odoratum* and *C. jacea* had a similar N content and C/N ratio, but root C content was 11% higher in *A. odoratum* than in *C. jacea* ( $P = 0.04$ ; Table 3.2). Lignin content was seven times higher in *C. jacea* than in *A. odoratum* roots ( $P = 0.03$ ). As a consequence, root lignin/N ratio was 7.7 times higher in *C. jacea* than in *A. odoratum* ( $P = 0.03$ ).

### **Species richness effect on root litter and compost**

There was a significant interaction between the effects of plant species richness and substrate composition on the percentage of root or compost C decomposed ( $P = 0.04$ ; Fig. 3.1a). This interaction was due to a different response between root litters and compost. When analysing the effect separately for root litters, we found that the percentage of root C decomposed increased significantly with species richness ( $P < 0.001$ ; Fig. 3.1a), for single as well as mixed litters, with parallel responses for the three types of root litter (species richness  $\times$  root litter:  $P = 0.92$ ). This indicates that plant species richness enhanced root decomposition through a soil ecosystem effect. On the contrary,

there was no significant effect of soil ecosystem associated with species richness on compost decomposition.

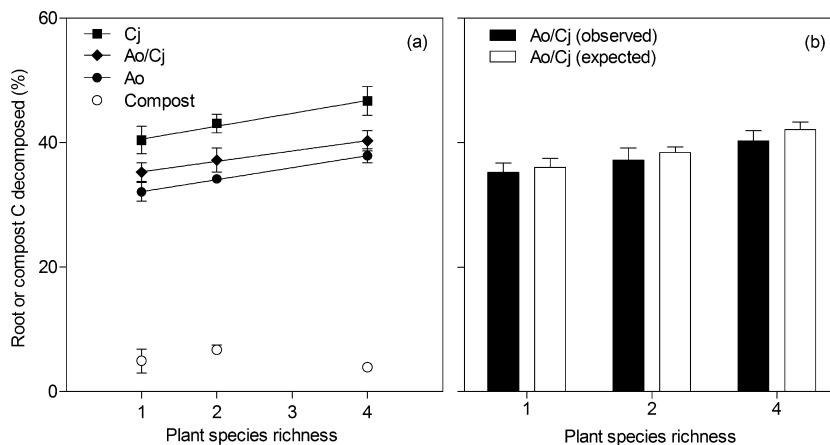
The rate of root decomposition significantly varied among root litters ( $P = 0.03$ ; Fig. 3.1a): root litter of *C. jacea* decomposed faster than that of *A. odoratum* and the root mixture of the two species (Ao/Cj), suggesting a possible negative litter mixing effect. Indeed, the decomposition rate of root mixture tended to be slower than expected from the decomposition of the single litters, but the difference was not statistically significant ( $P = 0.85$ ; Fig. 3.1b).

We further examined whether the positive soil ecosystem effect associated with greater species richness on root litter decomposition was related to species composition. The results showed that the species richness effect still occurred ( $P < 0.001$ ) after taking the effect of species composition into account (Fig. 3.2). Species composition did not significantly affect the decomposition of root litter (single litter or mixture) ( $P = 0.19$ ). We did not find an effect of species composition on compost decomposition ( $P = 0.09$ ).

## Discussion

Results of this study demonstrate that plant species richness accelerates the decomposition of root litter through a soil ecosystem effect. The rate of decomposition of mixed litter did not differ significantly from the average rate of decomposition of the single litters. The soil ecosystem effect associated with plant species richness persisted after species composition was taken into account. Moreover, the soil ecosystem effect resulting from increasing species richness was not modulated by the mixture of litter. These findings extend the literature on positive effects of species richness on decomposition of single leaf litters reported in grasslands (Hector et al., 2000; Spehn et al., 2005; Vogel et al., 2013) to decomposition of mixed root litters in grasslands. Importantly, these results indicate that soil C sequestration in diverse grassland communities, resulting from enhanced biomass input (Fornara and Tilman, 2008;

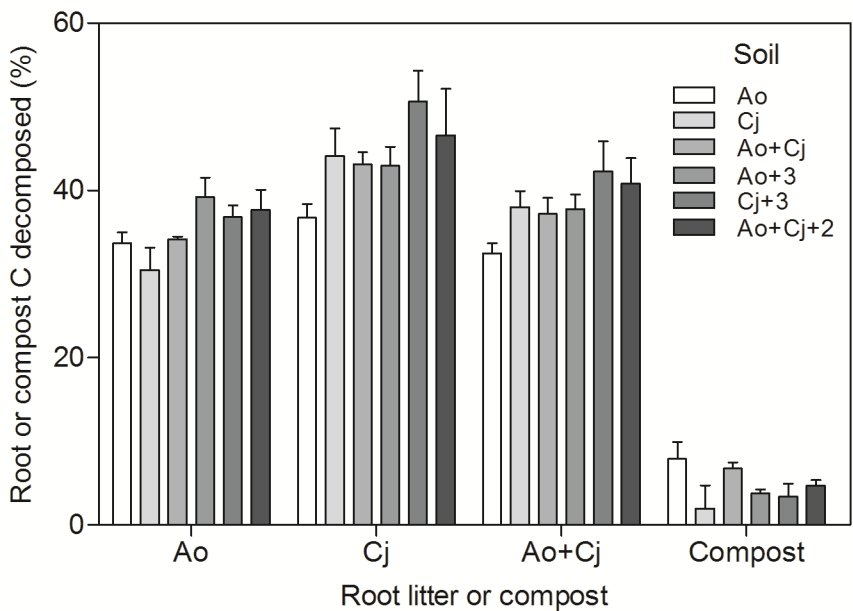




**Figure 3.1** Effects of plant species richness on decomposition of root litter and compost in the laboratory. (a) Soil ecosystem effect associated with plant species richness (x-axis) on C decomposed (%) of roots of *Anthoxanthum odoratum* (Ao), *Centaurea jacea* (Cj), the mixture of the two species (Ao/Cj) and compost. (b) Litter mixing effect on C decomposed (%) of the root mixture (Ao/Cj). Expected value is calculated from the root litters of single species. Data are means  $\pm$  SEM,  $n = 4$ .

Steinbeiss et al., 2008) can be partly counteracted by accelerated decomposition of the organic matter in soil.

The soil ecosystem effect on root litter decomposition may be attributed to the changes in soil microbiology and/or soil chemistry. Soil physical conditions such as soil structure and soil moisture content were identical during incubation, and can therefore be ruled out as possible causes for differences in decomposition rate. Several studies have shown that plant species richness increases soil microbial biomass (Chung et al., 2007; Eisenhauer et al., 2010; Zak et al., 2003). Since soil microorganisms rely on plant-derived residues entering the soil, the main driver for plant diversity-induced changes in soil microbial properties is increased organic matter input into soil, resulting from greater plant biomass production (Hooper et al., 2005). For example, De Deyn et al. (2011) and Zak et al. (2003) have demonstrated that plant species richness promotes the abundance of



**Figure 3.2** Effects of plant species composition (*i.e.* six soils) on decomposition of root litter and compost in the laboratory. Abbreviations refer to Table 3.1. Data are means  $\pm$  SEM,  $n = 4$ .

saprophytic fungi via changes in resource quantity. This mechanism may have operated in this study, where plant species richness enhanced plant productivity (Van Ruijven and Berendse, 2005, 2009).

Recent studies have suggested that plant species richness may enhance microbial biomass and activity through providing more diverse plant-derived resources (Eisenhauer et al., 2011; Meier and Bowman, 2008; Stephan et al., 2000). In the current study, *A. odoratum* and *C. jacea* were found to differ in root quality (Table 3.2). We may therefore expect greater decomposition in high-diversity plots if the above mechanism occurs. However, our results showed that mixing the two litters did not significantly affect decomposition of root litter to above or below the expected value calculated with Eq. 2, when accounting for the shares of the fast decomposing litter of *C. jacea* and the more slowly decomposing litter of *A. odoratum*. Collectively, our results indicated that diversity of root litters had

only a minor effect on litter decomposition if any. Moreover, plant diversity-induced changes in soil chemistry such as inorganic N availability may indirectly affect root decomposition through effects on microbial biomass and activity. Our results showed increased net N mineralization rates with species richness in a parallel laboratory experiment with the same soil samples (Cong et al., submitted). This indicates that inorganic N was not the limiting factor for soil microbe in this study, making this mechanism less likely.

Contrary to root litter, the decomposition of compost was not enhanced in soil from high diversity plots. This suggests the specie richness effect plays a key role in affecting the decomposition of relatively labile material (e.g. root litter) more than that of recalcitrant material such as compost. Soil microorganisms vary in their capacity of decomposing fresh organic matter and recalcitrant organic matter (Paul and Clark, 1989). Apparently, in this study, species richness may have particularly affected soil microorganisms specialized in the decomposition of fresh organic matter rather than those feeding on recalcitrant material.

In conclusion, we have shown that plant species richness enhances root litter decomposition via changes in soil ecosystem. The soil-mediated effect is not significantly modulated by the diversity of litter. More importantly, accelerated root decomposition may partly counteract enhanced biomass C input transformed into soil organic matter, thus reducing the soil C sequestration potential in diversified grassland communities.

## Acknowledgements

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# Chapter 4

## **Learning from nature: ecological intensification through intercropping enhances sequestration of soil carbon and nitrogen**

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Xingguo Bao, Fusuo Zhang and Wopke van der Werf*



*Photo by Fusuo Zhang*

### **Abstract**

Biodiversity enhances productivity and soil C and N sequestration in natural systems with perennial species and low N levels. Here, we demonstrate for the first time that mixed species cultivation increases soil C and N in an agricultural system with only annual plant species and high N input. After 7 years of rotations of maize, wheat and faba bean intercrops, soil organic C content was  $4\% \pm 1\%$  greater than in rotations of single crops, while total soil N content was  $11\% \pm 1\%$  greater in intercrop rotations than in rotations of sole crops. Root biomass was 23% greater in intercrops than in single crops, explaining C sequestration. These effects occurred in systems with and without legumes. A broad suite of complementarity effects contributed to N retention in intercrops. The results demonstrate that findings on biodiversity effects in natural systems are relevant to innovating agriculture.

## Introduction

Primary production in natural ecosystems is positively associated with plant diversity (Cardinale et al., 2012) while storage of soil C and N is on average greater in systems with more species (Fornara and Tilman, 2008; Steinbeiss et al., 2008). These effects result from functional complementarity between different species (Fornara and Tilman, 2008; Steinbeiss et al., 2008). Intercropping, i.e. the simultaneous cultivation of two or more crop species in the same field, is one of the approaches to establish greater plant diversity and functional complementarity within agroecosystems. Intercropping generally enhances productivity (Willey, 1979; Lithourgidis et al., 2011) and is a plausible strategy for ecological intensification<sup>1</sup> but its effects on soil functioning are largely unexplored. We postulate that intercropping may have benefits for soil functioning that are additive to the well-established positive effects of crop diversification through rotation and cover-cropping (McDaniel et al., 2014).

Despite a large intercropping literature, little information is available on the long-term effects of intercropping on the provision of soil ecosystem services, e.g. sequestration of C and N. Soil C and N levels are key quality parameters for agricultural soils because they are positively linked to the supply of nutrients to the crop, water-holding capacity, workability, and resistance to soil compaction, erosion and surface crusting (Weil and Magdoff, 2004). Aside from its effect on soil quality, enhanced C sequestration is also of interest to mitigate increasing atmospheric CO<sub>2</sub> concentrations (Lal, 2004).

Intercropping has been shown to achieve higher yields; the higher yields are attributed to complementarity between crop species in temporal and spatial patterns of resource acquisition (Hinsinger et al., 2011), facilitation (Zhang and Li, 2003; Hauggaard-Nielsen and Jensen, 2005), and/or reduction in the impacts of pests, diseases and weeds (Liebman

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<sup>1</sup> Defined as “the environmentally friendly replacement of anthropogenic inputs and/or enhancement of crop productivity, by including regulating and supporting ecosystem services management in agricultural practices” (Bommarco et al., 2013).

and Dyck, 1993; Trenbath, 1993; Zhu et al., 2000). The literature is consistent on these positive effects of intercropping, but it can be postulated that intercropping would diminish soil nutrient levels in the longer term because of the greater plant productivity and hence greater nutrient removal through harvesting in mixed systems compared to single crop systems (Zhang and Li, 2003).

The positive effect of enhanced species diversity on soil C and N storage in natural grasslands has been attributed to enhanced belowground input of organic matter derived from a greater root production (Fornara and Tilman, 2008; Steinbeiss et al., 2008). The greater belowground biomass production is, for example, associated with the presence of complementary functional plant groups, such as C4 grasses and legumes (Fornara and Tilman, 2008). Complementarities between species can occur in intercropping systems, too (Lithourgidis et al., 2011), and recent studies suggest greater input of C into the soil through root residues (Ghosh et al., 2006; Yang et al., 2010; Li et al., 2011b). However, agroecosystems differ in many respects from natural systems, for which the effects of diversity on productivity and sequestration of C and N have been well documented (Hooper et al., 2005; Tilman et al., 2006). First, they consist mainly of rotated annuals whereas natural ecosystems are dominated by perennials. The role of soil pathogens can be mitigated by rotation (Peters et al., 2003) and roots of annuals decompose faster than those of perennials (Wardle et al., 1997). Furthermore, aboveground residues are partly or wholly removed, reducing the input of litter into the soil. Finally, high N inputs in agroecosystems can suppress biological N fixation by legumes (Salvagiotti et al., 2008). It is therefore impossible to extrapolate the findings in natural grasslands to agroecosystems.

Candidate mechanisms for enhanced N storage through intercropping are, first, enhanced biological N fixation by legumes when intercropped with cereals (Li et al., 2009) and, second, improved N capture in mixed crops as a result of complementarity in foraging strategies in space (soil profile) and time (growth period of the crop) (Vandermeer, 1989; Li et al., 2005; Lithourgidis et al., 2011). On the other hand, intercropping may have greater crop N removal resulting from higher yields as compared to single cropping



systems. There are no reports on long-term effects on the storage of C and N in the soil under intercropping. Some work in this area has been done (Myaka et al., 2006; Snapp et al., 2010), but the duration of those studies was too short to convincingly demonstrate sequestration of organic C and N.

In the studies reported here, we determined soil C and N content after 7 years of intercropping, as compared to 7 years of single crops, where both the intercrops and single crops were grown in rotation. We further determined root biomass, as a proxy for belowground productivity, in two-species intercrops of maize, wheat and faba bean and compared it to belowground productivity in the single crop systems. We tested the following hypotheses: 1) Intercropping enhances belowground root biomass; 2) The enhanced root C input in intercropping systems results in soil C sequestration over the long term; and 3) As a result of enhanced C sequestration and species complementarity in N acquisition strategies, intercropping systems sequester more soil N than single cropping systems.

Our key findings are that (1) intercropping enhanced soil C and N across species combinations, (2) root biomass is substantially higher in intercrops as compared to single crops, providing a plausible explanation for the increase in soil C, and (3) several mechanisms contribute to N retention.

## Materials and methods

The formulated hypotheses were tested in two experiments. First, sequestration of C and N in the soil was determined in a long-term experiment that started 7 years prior to the current sampling of the intercropping and single cropping systems (Li et al., 2007). Three crop species (maize, *Zea mays* L.; wheat, *Triticum aestivum* L.; and faba bean, *Vicia faba* L.) were grown both as single crops and as two-way intercrops in a 2-year rotation (Fig. 4.1). Secondly, two repeated short-term field experiments (2008 and 2011) were sampled to quantify root biomass in intercropping versus single cropping systems. All experiments

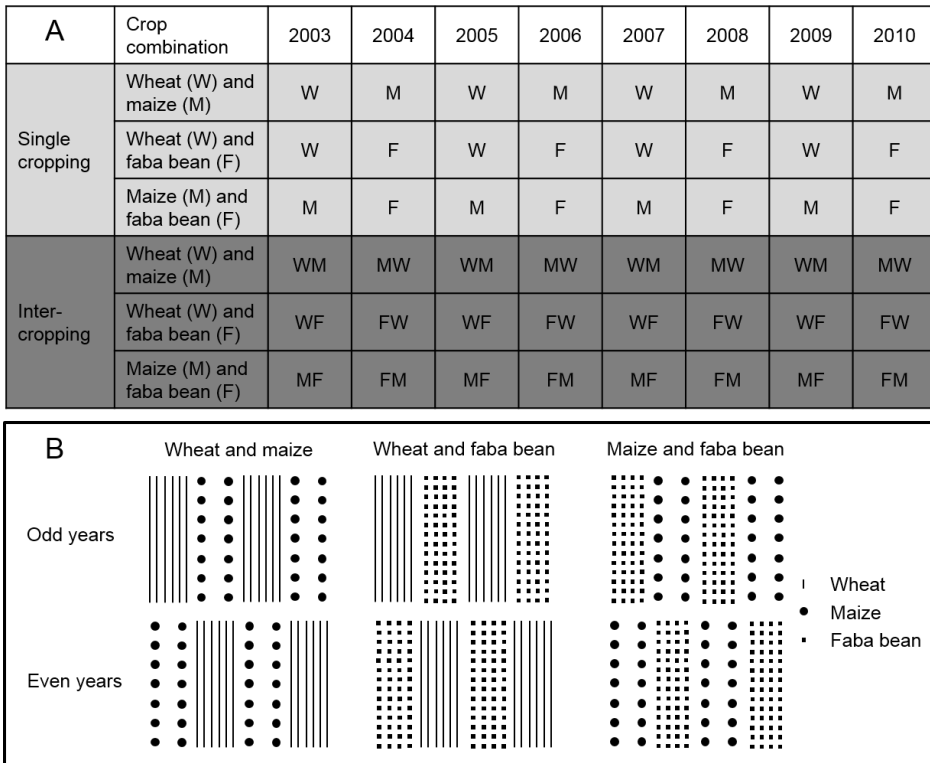
were conducted at Baiyun Experimental station (Wuwei, Gansu, China) and identically managed.

### ***Sequestration of soil C and N***

A long-term field experiment was established at Baiyun Experimental Station (38°37'N, 102°40'E) in Gansu province, Northwest China in 2003. Average annual temperature at the site is 8.9 °C. Climate is arid with a total yearly rainfall of  $168 \pm 8$  mm and potential evaporation of 2021 mm. Soil pH was 8.2, organic C content 11.42 g kg<sup>-1</sup>, total N 1.28 g kg<sup>-1</sup>, and P-Olsen 21.2 mg kg<sup>-1</sup> in 2003. Soil texture is a sandy loam from 0-71 cm soil depth, clay loam from 71-106 cm, silty clay loam from 106-144 cm, and sand below. The field experiment was laid out as a completely randomized block design with three replicates.

Six cropping systems were compared: three 2-year single crop rotations (i.e. maize/wheat, maize/faba bean and wheat/faba bean), and three intercrops of the same species combinations whereby the crop species were grown in narrow strips and rotation was conducted at the level of the strips (Fig. 4.1A and B). Maize/wheat intercrop was planted in 160 cm-wide strips consisting of an 80 cm-wide maize strip with two rows of maize at 40 cm row distance, and an 80 cm-wide wheat strip with six rows of wheat at 13.3 cm row distance. The maize/faba bean and wheat/faba bean intercrops had the same overall design, but with an 80 cm-wide band of faba bean with four rows at 20 cm row distances. Row distance and plant distance in single cropping were the same as in intercropping. Thus, all intercropping combinations are arranged in a replacement design.

Each plot received 225 kg ha<sup>-1</sup> N as urea and 40 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as triple superphosphate. Two thirds of N fertilizer and all P fertilizer were incorporated as basal fertilizer into the topsoil with moldboard tillage before sowing and the remainder was top-dressed in all plots at wheat flowering. Single crops of wheat and faba bean received flood irrigation five times during the growing season, while single maize and intercrops with maize were irrigated two more times afterwards to meet the water demand of maize (Fig. S4.1).



**Figure 4.1** Overview of the six crop systems that were compared in this study, comprising three rotations of single crops and three rotations of intercrops (A). The intercrops are grown in a “small rotation” in which the position of the crop species is swapped yearly (B). The experiment was started in 2003 and soil samples were taken after seven years, in 2010.

Soil samples were collected using an auger from five depths at 20 cm intervals till 1 m depth in July 2010. Three soil samples per plot were taken in single crop plots and bulked per depth. To cover the designed heterogeneity within intercrop plots, nine samples were taken per plot: three were in the middle between two rows of one species, three were in the middle between two rows of the other species, and three were in the middle between rows of the two different species. Soil samples were bulked per plot, transported to the lab, air-dried for 3 days, and sieved (1 mm mesh) to remove visible plant material. To remove inorganic C, the samples were treated with HCl prior to analysis (Midwood and Boutton 1998). The acid-treated soil samples were ground in a ball mill. Soil organic C

and N contents were determined twice using C/N analysers in two different chemical laboratories: C/N analyser (Vario Macro, Elementar, Germany) at the Stable Isotope Lab of China Agricultural University, and C/N isotope ratio spectrometer (PDZ Europa Integra, Cheshire, United Kingdom) at the Stable Isotope Facility of the University of California. Soil  $\delta^{15}\text{N}$  was measured only in the latter. The results on SOC and STN contents were pooled for data analysis because systematic differences between the two determinations were not found and pooling allowed for greater confidence in the results. Part of the results on SOC (0-20 cm soil depth) were further validated against results from wet chemical oxidation (Kurmies) which showed exactly the same trends as the results from the C/N analysers. Soil bulk density was determined using 100 cm<sup>3</sup> cylinders. Three replicate samples (0-20 cm and 20-40 cm depths) were taken both in single cropping and intercropping systems. Soil samples were oven-dried (105°C) for 3 days before weighing.

Soil Organic C, STN, C/N ratio and  $\delta^{15}\text{N}$  throughout the soil profile were analysed using four-way repeated-measures ANOVA (SPSS, 17.0) with depth as within-subject factor, and block, cropping system and crop combination as between-subjects factors. Separate analyses on these variables were made per each soil layer using three-way ANOVA (SPSS, 17.0), with block as a random factor, and cropping system and crop combination as fixed factors.

### ***Above- and below-ground overyielding***

Field experiments were established at Baiyun Experimental station in 2008 and 2011, respectively, to measure aboveground and belowground plant biomass. A randomized block design was used in both years, with three replicates in 2008 and five replicates in 2011. The experimental treatments included single crops of maize, wheat and faba bean and two intercropping systems: maize/wheat and maize/faba bean in a replacement design. The experiment in 2011 was identical in design to the 7-year field experiment, while the design in 2008 was similar, but with two differences: (1) Each plot received 75 kg/ha P<sub>2</sub>O<sub>5</sub> instead of 40 kg/ha P<sub>2</sub>O<sub>5</sub>, and (2) the maize/wheat intercrop was planted in

alternating 150 cm wide strips consisting of a 78 cm wide maize band with two maize rows at 39 cm distance, and a 72 cm wide band of wheat with 6 rows at 12 cm row distance. The maize/faba bean intercrop was planted in 120 cm wide strips that consisted of an 80 cm wide maize strip with two rows at 40 cm row distance, and a 40 cm wide faba bean strip with two faba bean rows.

Aboveground biomass was determined by harvesting an intercropping strip at maturity of the crop. The area harvested in intercropping systems was identical to that harvested in the single cropping systems. Root biomass was determined from monoliths (Böhm, 1979) taken at the time of maximum root biomass: mid June for wheat and faba bean, and mid-August for maize. Briefly, trenches were dug perpendicular to the crop rows in each plot. Soil monoliths (40 cm length  $\times$  20 cm width  $\times$  20 cm depth) were then taken using an aluminium box along a 1 m soil profile, resulting in a stack of five monoliths in each plot. Soil was gently rinsed from the roots and roots were collected on a 1-mm sieve. Roots of wheat, faba bean and maize were separated on the basis of differences in colour and morphology and dried at 60°C for 3 days. A subsample of roots was taken for root C and N contents using vario MACRO Elemental Analyser (Elementar Company, Germany). Root biomass C and N were calculated by multiplying root biomass by root C and N concentrations.

Two measures were used to evaluate the effect of intercropping on aboveground and belowground productivity: Land equivalent ratio (LER) (Mead and Willey, 1980) and expected yield. LER was calculated as:

$$\text{LER} = \frac{Y_1}{M_1} + \frac{Y_2}{M_2}$$

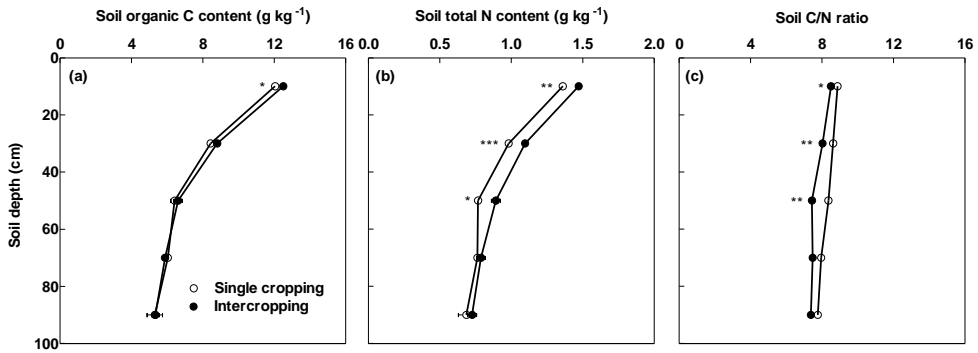
where  $Y_1$  is biomass density or biomass C, N ( $\text{g m}^{-2}$ ) of species 1 in intercrop, and  $M_1$  its biomass density or biomass C, N ( $\text{g m}^{-2}$ ) in single crop, and *vice versa* for species 2. Expected yield in intercrop is calculated as the yield of a species in single crop multiplied by the ratio of its densities in mixed and single crop. If the relative density of species 1 is  $a$ , then expected yield of species 1 is calculated as  $Y_e = aM_1$  and expected yield of species 2 as  $(1-a)M_2$ .

Total aboveground and belowground biomass as well as total root biomass C and N over the 1 m profile were analysed using three-way repeated-measures ANOVA, with year as within-subject factor, and cropping system and block as fixed factors. Two-way ANOVA was used to analyse the effects of cropping system and crop species combination on root biomass, root biomass C and N in each year as the first analysis indicated significant year  $\times$  cropping system interaction. Data on root biomass C and N at five depths were analysed using four-way repeated-measures ANOVA (SPSS, 17.0) with depth as within-subject factor, and block, cropping system (expected vs. observed) and crop combination as between-subjects factors. Root biomass C and N at each depth were analysed using three-way ANOVA (SPSS, 17.0) with block as a random factor, and cropping system (expected vs. observed) and crop combination as fixed factors.

## Results

### ***Sequestration of soil C and N***

Soil organic C content (SOC) in the long-term experiment differed significantly ( $P = 0.03$ ) between single cropping and intercropping systems averaged over crop species combinations:  $12.1 \pm 0.13$  versus  $12.5 \pm 0.15$  g kg<sup>-1</sup> in the top 20 cm of the soil profile (i.e. a difference of 4%) (Fig. 4.2A and Table S4.1), and  $10.2 \pm 0.12$  versus  $10.7 \pm 0.11$  g kg<sup>-1</sup> (a difference of 3%) in the top 40 cm ( $P = 0.04$ ; Table S4.2). From 40-100 cm depth, SOC was similar in intercropping and single cropping systems. Soil total N content (STN) differed by 8% in the top 20 cm ( $1.36 \pm 0.0$  versus  $1.47 \pm 0.01$  g kg<sup>-1</sup>; Fig. 4.2B and Table S4.1) and by 11% in the upper 60 cm (Table S4.2), i.e. the effect of intercropping on STN was more than two times as large as that on SOC. All effects of intercropping on SOC and STN were independent of which two crop species were combined (Tables S4.1 and S4.2). Soil C/N ratio across the top 60 cm of the soil profile was significantly decreased by intercropping (Fig. 4.2C). Combining these changes in C and N content with the soil bulk density of 1.44 g cm<sup>-3</sup> (equal in single cropping and intercropping systems; Fig. S4.2), we found a difference between the rates of sequestration in the top 20 cm soil layer of



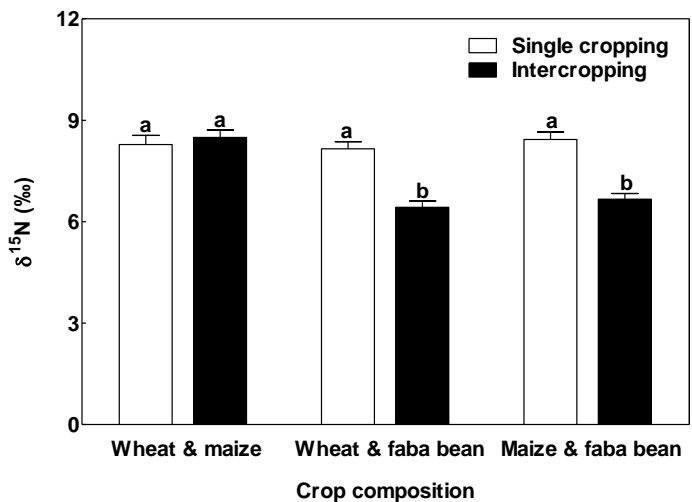
**Figure 4.2** Soil organic C (A), soil total N (B) and C/N ratio (C) across a 1 m deep soil profile, averaged over three rotated intercropping systems (maize/wheat, maize/faba bean and wheat/faba bean) (closed symbols) and single crop rotations with the same species (open symbols) in July 2010, after 7 years in a long-term experiment. Data are means  $\pm$  SEM,  $N = 9$ . Asterisks refer to significant differences between single cropping and intercropping per each depth: \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

intercropping and single cropping plots of  $184 \pm 86 \text{ kg C ha}^{-1} \text{ year}^{-1}$  and  $45 \pm 10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ .

Soil  $\delta^{15}\text{N}$  was lower in long-term intercropping systems with faba bean than in the maize/wheat intercropping system ( $P = 0.001$ ); it was also lower in the rotated intercrops with faba bean than in the corresponding single crop rotations ( $P = 0.003$ ) (Fig. 4.3). As biological N fixation by legumes is associated with a lowering of the  $\delta^{15}\text{N}$  signature of the soil (Högberg 1997), this result indicates a higher biological N fixation by legumes in intercropping than single cropping systems with faba bean.

### **Above- and below-ground overyielding**

In the short-term experiments, intercropping systems showed overyielding. This was characterized by land equivalent ratios for grain yield from 1.21 to 1.37 (Table S4.3), indicating that 1.21 to 1.37 times the land surface area would be needed to produce the same yield in single as in intercropping systems. The aboveground biomass produced in intercropping systems was 13% to 23% greater than expected from single cropping

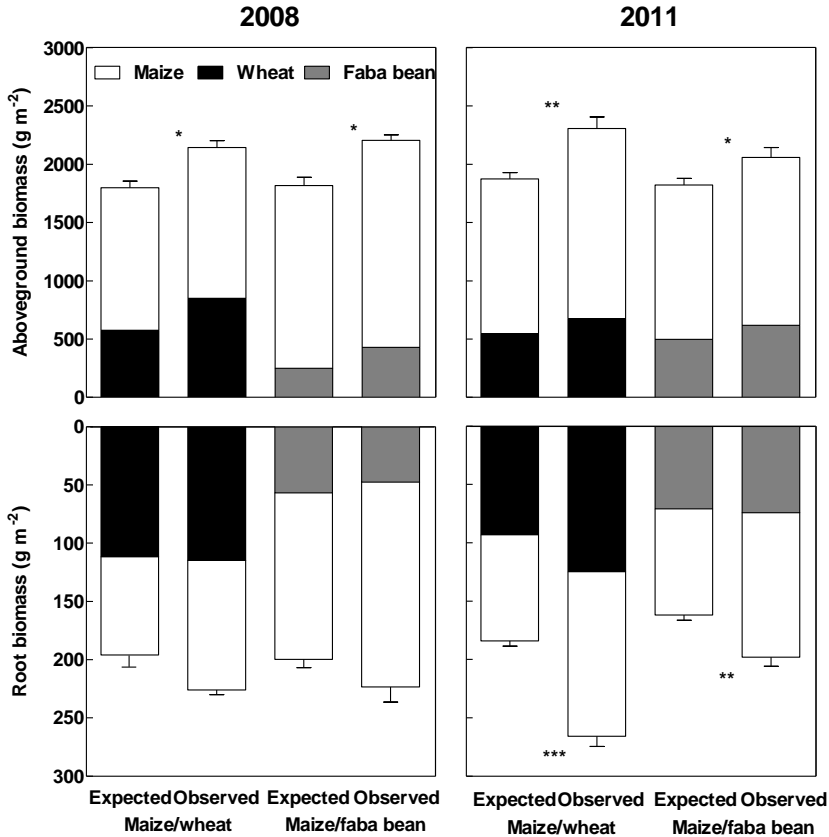


**Figure 4.3**  $\delta^{15}\text{N}$  over the whole soil profile (0-100 cm) in six cropping systems after 7 years in a long-term experiment. Data are means  $\pm$  SEM,  $N = 3$ . Means with the same letter do not differ significantly within a crop combination ( $P = 0.05$ ).

biomass production (Fig. 4.4). Land equivalent ratio for belowground biomass ranged from 1.10 to 1.45 (Table S4.3). The maximum standing root biomass was on average 23% greater in intercrops than expected from belowground biomass measured in single crops (Fig. 4.4).

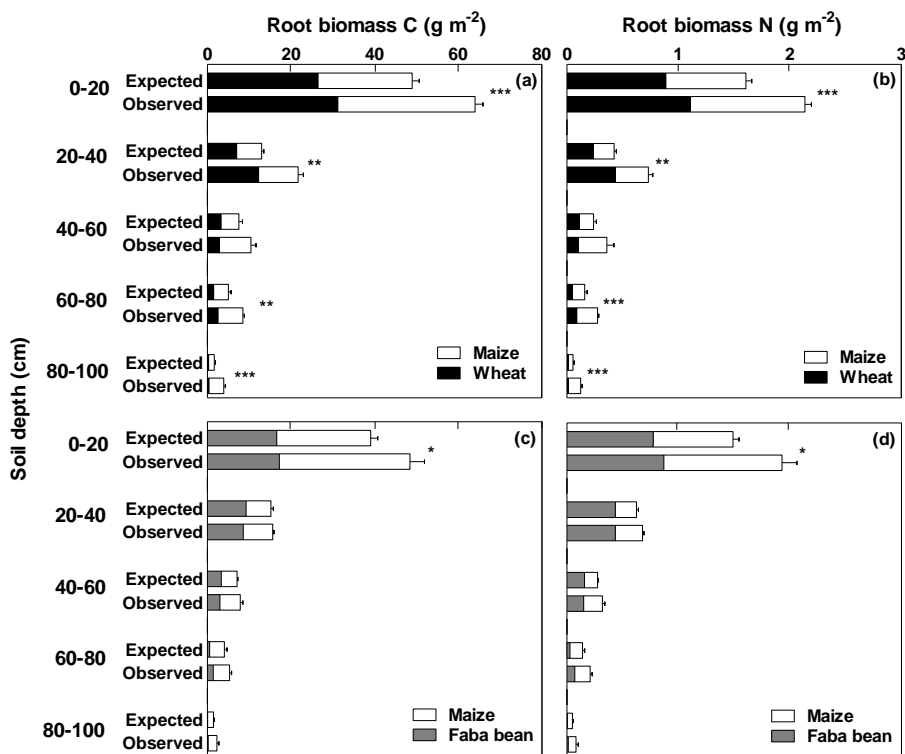
Total root biomass C and N in intercropping systems exceeded the expected in both tested species combinations (maize/wheat and maize/faba bean) at all depths (Fig. 4.5). Significant differences were found across the whole soil profile in maize/wheat intercrops, whereas the difference between expected and observed root biomass C and N in maize/faba bean intercrops was only significant in the upper 20 cm. In the maize/faba bean combination, the increase in root biomass C and N was found in the topsoil only, and could be attributed mainly to maize (Fig. 4.5C and D) whereas in maize/wheat intercropping there was also a contribution from wheat in the top 40 cm.





**Figure 4.4** Comparison of expected and observed aboveground and belowground biomass in intercropping combinations of maize with wheat or faba bean. Expected yield was calculated from biomass of the corresponding single crops. Data are means  $\pm$  SEM ( $N = 3$  in 2008;  $N = 5$  in 2011). Asterisks refer to significant differences between expected and observed values \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ . Aboveground biomass in 2008 was previously reported (Li et al., 2011c).

Intercropping did not significantly affect root C and N concentrations in any of the three crop species (Fig. S4.3). Root C/N ratios were 32 for maize and 30 for wheat, and 22 (significantly lower than in the other species at  $P < 0.05$ ) for faba bean.



**Figure 4.5** Standing root biomass C (A, C) and N (B, D) in five soil layers in maize/wheat (A, B) and maize/faba bean (C, D) intercrops rotations in 2011 versus expected values based upon rotations of the same species as single crop. Expected root biomass C and N were calculated from results of the corresponding single crops. Data are means  $\pm$  SEM,  $N = 5$ . Asterisks refer to significant differences between expected and observed values at each depth. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ .

## Discussion

Intercropping enhanced soil C and N levels. The enhanced soil C levels are attributed to a greater root biomass production, and enhanced soil N levels must probably be attributed to a combination of greater C input and complementary N acquisition strategies of intercropped species. In contrast to what has been reported so far on natural ecosystems, soil N effects did not depend on the presence of a legume. Thus, we demonstrated that positive effects of biodiversity on ecosystem functioning reported in N-limited perennial grassland ecosystems are paralleled in N-rich annual intercropping agro-ecosystems from which most of the biomass produced is removed at harvest. These here-to-fore unrecognized soil ecosystem services amplify well-known advantages of mixed crop systems in terms of productivity, production risk mitigation and suppression of pests and diseases (Vandermeer, 1989; Trenbath, 1993; Lithourgidis et al., 2011).

We identified a significant difference in sequestered SOC between plots cultivated with 2-year rotations of intercropped maize, wheat or faba bean, and those cultivated with rotated single crops over a period of 7 years. The difference was significant in the top 20 cm of the soil (Fig. 4.2A), and amounted to a  $4\% \pm 1\%$  greater storage, equivalent to approximately  $184 \pm 86 \text{ kg C ha}^{-1} \text{ year}^{-1}$ . The amount of sequestered C was similar for all three crop combinations tested. This C sequestration is, in part, related to an increase in root biomass, which was on average 23% greater in intercropping systems than expected from observations in single cropping systems (Fig. 4.4). Intercropping systems that consist of species with differing growing periods can intercept more light than single cropping systems, resulting in greater biomass and yield (Zhang et al., 2008). This mechanism for temporal niche differentiation is likely to result in greater belowground C input as crop species maintain a functional balance between the shoot and the root system (Poorter et al., 2012).

Intercropping systems also sequestered more STN as compared to rotations of single crops (Fig. 4.2B). The difference amounted to an  $11\% \pm 1\%$  increase in N storage,

equivalent to  $45 \pm 10 \text{ kg N ha}^{-1} \text{ year}^{-1}$ . The first plausible causal mechanism for the increase in STN is stoichiometry: proportional to the increase in belowground C input, also more N was found in roots. This is evident in our finding that there was more N in roots in intercropping than in single cropping systems (Fig. 4.5B and D). But there must be more to explain the disproportionate increase in soil N compared to soil C.

The lower soil  $\delta^{15}\text{N}$  in intercrop rotations with faba bean in our study indicates that increased biological N fixation contributed to the enhanced N storage (Fig. 4.3). Previous studies demonstrated that intercropping legumes with cereal crops enhances biological N fixation of legumes, even under high N fertilization level (Li et al., 2009). Intercropping with legumes also enhances N acquisition by cereals, first because competition for soil mineral N is reduced (Jensen, 1996b), and secondly because some of the N fixed by legumes can be transferred to cereals through root exudation (Jensen, 1996a). A lowering of  $\delta^{15}\text{N}$  would also occur if denitrification was reduced, as gaseous losses discriminate against the heavier  $^{15}\text{N}$  isotope. This possibility cannot be ruled out, and is even plausible, as intercropping with cereals tends to lower soil nitrate levels in intercrops with faba bean (Li et al., 2005) but further work is needed to quantify these losses.

One of the key findings is that N accumulation was similar in systems with and without faba bean. As there was no change in  $\delta^{15}\text{N}$  in the maize/wheat intercropping system, losses that do not discriminate against  $^{15}\text{N}$  must explain the enhanced N storage. The key mechanism is probably a reduction in N leaching, the main N loss pathway in the North China Plain (Ju et al., 2009). Reduced leaching is partly a consequence of functional complementarity between crop species in the location and timing of N uptake across the soil profile: The combined root systems of the two intercropped species can intercept nitrate that would otherwise be lost from the profile due to greater root biomass (Table S4.3; Fig. 4.4), presence of active roots during a longer part of the growing season (Fig. S4.1) and greater root biomass in deeper layers (Fig. 4.5). Additionally, N recycling within the growing season is probably enhanced: after the harvest of the early maturing crops (wheat and faba bean) their root litter starts decomposing, releasing mineral N into the

soil. Maize roots can proliferate in the N rich top layers (Drew and Saker 1975) that were previously colonized by wheat and faba bean roots, and prevent the mineralised N from being lost from the soil system. Our findings extend earlier indications for higher N retention in more diverse natural grasslands (Tilman et al., 1996; Hooper and Vitousek, 1997; Tilman et al., 1997a) to high N input agricultural systems.

The potential benefits of intercropping for improving long-term soil fertility by enhanced C and N sequestration may be more important than the possible contribution to greenhouse gas mitigation. Agricultural management practices such as no tillage, cover crops and complex rotations with deeper roots can enhance the rate of C sequestration by 50 to 500 kg C ha<sup>-1</sup> year<sup>-1</sup> (West and Post, 2002; Lal, 2004). The effect of intercropping on soil C storage is within this range, and is thus comparable in potential to other proposed agricultural mitigation measures. The contribution to greenhouse gas mitigation in China is, however, minor. Assuming that one third of China's cropland may be used for intercropping (Zhang and Li 2003), the C sequestration potential of intercropping in China is estimated to be 7.4 Tg C year<sup>-1</sup>, which accounts for only 0.4 % of the total greenhouse gas emissions (6100 Tg CO<sub>2</sub>-eq) in China in 2004 (Zhang et al., 2013b).

Biodiversity effects on soil C and N storage in natural grasslands are associated with functional complementarity between plant species, e.g. between legumes that can fix N, and C4 grasses that can use this N efficiently to produce biomass (Fornara and Tilman, 2008). In our study, not only did we find the functional complementarity between faba bean (legume, early growing season) and maize (C4, late growing season), but we also found functional complementarity between wheat (C3, early growing season) and maize (C4, late growing season) as well as wheat (C3, early growing season) and faba bean (legume, early growing season). All tested intercrop systems showed similar increases in SOC and STN. Considering the role of functional complementarity in overyielding of intercropping systems (Lithourgidis et al., 2011) and in soil C and N sequestration (this study), we suggest that those systems that have the highest production can also have the greatest benefits for soil functioning in the long term, i.e. by organic matter sequestration,

reducing N leaching, and curbing gaseous N losses. This conclusion parallels insights derived from natural systems in which functional complementarity in N uptake between species enhances productivity and reduces N losses (Tilman et al., 1996; Hooper and Vitousek, 1997; Tilman et al., 1997a).

Concluding, intercropping increased C and N storage in the soil profile while it also enhanced yields, suggesting a win-win scenario for intercropping: short-term gains in yield are accompanied by long-term gains in soil quality. The accumulation of soil C and N may further benefit soil fertility and intercropping system productivity in the long term. Additional wins consist of mitigation of greenhouse gas emissions and nitrate leaching.

## Acknowledgements

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Supporting Information

**Table S4.1** Soil organic C (SOC) and total N (STN) (g kg<sup>-1</sup>) at five depths in six cropping systems (mean ± SEM, N = 3). P values are from three-way ANOVA with block as random factor, and cropping system (single cropping vs. intercropping) and crop species combination as fixed factors. Significant effects (P < 0.05) are shown in bold.

Variables	Depth (cm)	SED	Maize-wheat		Wheat-faba bean		Maize-faba bean		Across crop combinations		P values		
			Single cropping	Intercropping	Single cropping	Intercropping	Single cropping	Intercropping	Single cropping	Intercropping	Cs <sup>*</sup>	Cc <sup>†</sup>	Cs × Cc
SOC	0-20	0.39	11.85 ± 0.20	12.86 ± 0.09	12.05 ± 0.31	12.61 ± 0.18	12.25 ± 0.21	12.04 ± 0.29	12.05 ± 0.13	12.50 ± 0.15	<b>0.03</b>	0.61	0.06
	20-40	0.53	8.32 ± 0.35	8.68 ± 0.41	8.27 ± 0.10	9.04 ± 0.33	8.70 ± 0.36	8.72 ± 0.21	8.43 ± 0.15	8.81 ± 0.17	0.16	0.80	0.49
	40-60	0.75	6.75 ± 0.31	6.55 ± 0.31	6.15 ± 0.50	6.51 ± 0.35	6.34 ± 0.35	6.79 ± 0.58	6.41 ± 0.20	6.62 ± 0.21	0.58	0.75	0.72
	60-80	0.40	6.11 ± 0.18	5.96 ± 0.27	5.93 ± 0.15	5.34 ± 0.21	6.09 ± 0.29	6.35 ± 0.17	6.05 ± 0.10	5.88 ± 0.17	0.40	0.07	0.43
	80-100	0.79	5.29 ± 0.50	5.14 ± 0.10	4.80 ± 0.67	5.00 ± 0.37	5.84 ± 1.09	5.88 ± 0.38	5.31 ± 0.40	5.34 ± 0.20	0.94	0.15	0.93
STN	0-20	0.05	1.31 ± 0.02	1.47 ± 0.01	1.38 ± 0.06	1.46 ± 0.03	1.39 ± 0.01	1.49 ± 0.02	1.36 ± 0.02	1.47 ± 0.01	<b>0.00</b>	0.35	0.55
	20-40	0.05	0.93 ± 0.05	1.06 ± 0.03	0.96 ± 0.01	1.12 ± 0.03	1.04 ± 0.01	1.11 ± 0.02	0.98 ± 0.02	1.10 ± 0.02	<b>0.00</b>	0.07	0.32
	40-60	0.08	0.80 ± 0.03	0.83 ± 0.03	0.77 ± 0.04	0.91 ± 0.06	0.74 ± 0.05	0.93 ± 0.07	0.77 ± 0.02	0.89 ± 0.03	<b>0.01</b>	0.88	0.29
	60-80	0.07	0.79 ± 0.04	0.76 ± 0.05	0.72 ± 0.00	0.73 ± 0.04	0.77 ± 0.03	0.87 ± 0.04	0.76 ± 0.02	0.79 ± 0.03	0.39	0.09	0.30
	80-100	0.10	0.68 ± 0.06	0.68 ± 0.01	0.61 ± 0.07	0.69 ± 0.05	0.76 ± 0.14	0.81 ± 0.06	0.69 ± 0.05	0.73 ± 0.03	0.40	0.09	0.78

<sup>\*</sup>Cs: cropping system (single cropping vs. intercropping); <sup>†</sup>Cc: crop species combination.

**Table S4.2** Accumulated soil organic C (SOC) and total N (STN) (g kg<sup>-1</sup>) from the top of the profile down to five different depths in six cropping systems (means ± SEM, N = 3). P values are from three-way ANOVA with block as random factor, and cropping system (single cropping vs. intercropping) and crop species combination as fixed factors. Significant effects (P < 0.05) are shown in bold.

Variables	Depth (cm)	SED	Maize-wheat		Wheat-faba bean		Maize-faba bean		Across crop combinations		P values	
			Single cropping	Intercropping	Single cropping	Intercropping	Single cropping	Intercropping	Single cropping	Intercropping	Cs <sup>*</sup>	Cc <sup>†</sup> Cs x Cc
SOC	0-20	0.39	11.85 ± 0.20	12.86 ± 0.09	12.05 ± 0.31	12.61 ± 0.18	12.25 ± 0.21	12.04 ± 0.29	12.05 ± 0.13	12.50 ± 0.15	<b>0.03</b>	0.61 0.06
	20-40	0.53	10.09 ± 0.26	10.77 ± 0.24	10.16 ± 0.19	10.83 ± 0.13	10.48 ± 0.25	10.38 ± 0.21	10.24 ± 0.12	10.66 ± 0.11	<b>0.04</b>	0.94 0.17
	40-60	0.75	8.98 ± 0.25	9.36 ± 0.23	8.82 ± 0.25	9.39 ± 0.20	9.10 ± 0.27	9.18 ± 0.27	8.96 ± 0.13	9.31 ± 0.11	0.12	0.97 0.64
	60-80	0.69	8.26 ± 0.14	8.51 ± 0.11	8.10 ± 0.19	8.37 ± 0.17	8.35 ± 0.27	8.47 ± 0.22	8.23 ± 0.10	8.45 ± 0.08	0.21	0.85 0.92
	80-100	0.82	7.66 ± 0.14	7.84 ± 0.10	7.44 ± 0.18	7.70 ± 0.21	7.85 ± 0.43	7.96 ± 0.13	7.65 ± 0.14	7.83 ± 0.08	0.32	0.33 0.94
STN	0-20	0.05	1.31 ± 0.02	1.47 ± 0.01	1.38 ± 0.06	1.46 ± 0.03	1.39 ± 0.01	1.49 ± 0.02	1.36 ± 0.02	1.47 ± 0.01	<b>0.00</b>	0.35 0.55
	20-40	0.05	1.12 ± 0.03	1.26 ± 0.01	1.17 ± 0.03	1.29 ± 0.02	1.22 ± 0.01	1.30 ± 0.00	1.17 ± 0.02	1.28 ± 0.01	<b>0.00</b>	0.06 0.44
	40-60	0.08	1.01 ± 0.03	1.12 ± 0.01	1.04 ± 0.03	1.17 ± 0.03	1.06 ± 0.01	1.17 ± 0.02	1.04 ± 0.01	1.15 ± 0.01	<b>0.00</b>	0.20 0.89
	60-80	0.07	0.96 ± 0.01	1.03 ± 0.01	0.96 ± 0.02	1.06 ± 0.03	0.99 ± 0.01	1.10 ± 0.02	0.97 ± 0.01	1.06 ± 0.01	<b>0.00</b>	0.13 0.63
	80-100	0.08	0.90 ± 0.02	0.96 ± 0.00	0.89 ± 0.01	0.98 ± 0.03	0.94 ± 0.04	1.04 ± 0.00	0.91 ± 0.01	0.99 ± 0.01	<b>0.00</b>	<b>0.03</b> 0.54

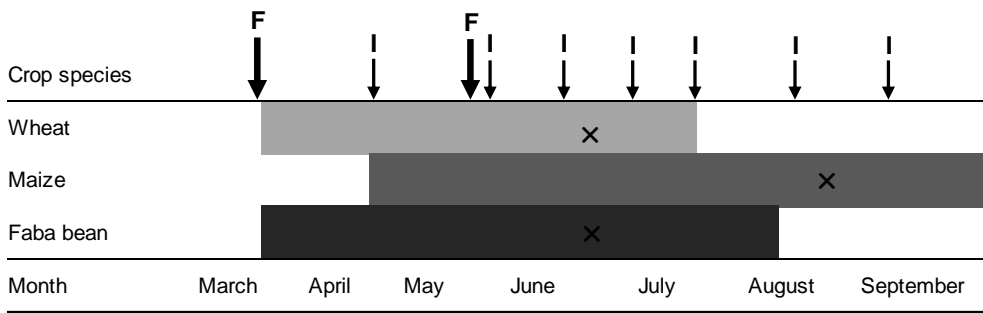
<sup>\*</sup>Cs: cropping system (single cropping vs. intercropping); <sup>†</sup>Cc: crop species combination.



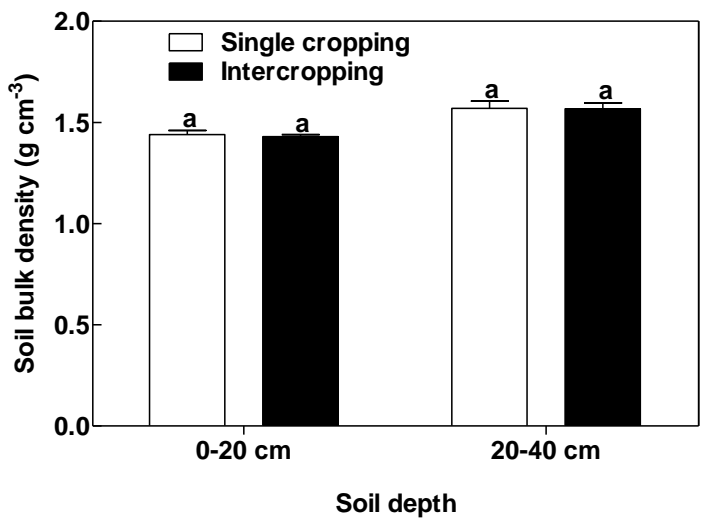
**Table S4.3** Land equivalent ratio of aboveground biomass, grain yield, standing root biomass, standing root biomass C and N in maize/wheat and maize/faba bean intercropping systems in 2008 and 2011

Year	Variables	Maize/wheat intercrop	Maize/faba bean intercrop
2008	Aboveground biomass <sup>*</sup>	1.32	1.33
	Grain yield <sup>†</sup>	1.26	1.22
	Root biomass	1.17	1.10
	Root biomass C	1.15	1.12
	Root biomass N	1.17	1.21
2011	Aboveground biomass	1.23	1.19
	Grain yield	1.21	1.37
	Root biomass	1.45	1.24
	Root biomass C	1.43	1.18
	Root biomass N	1.46	1.27

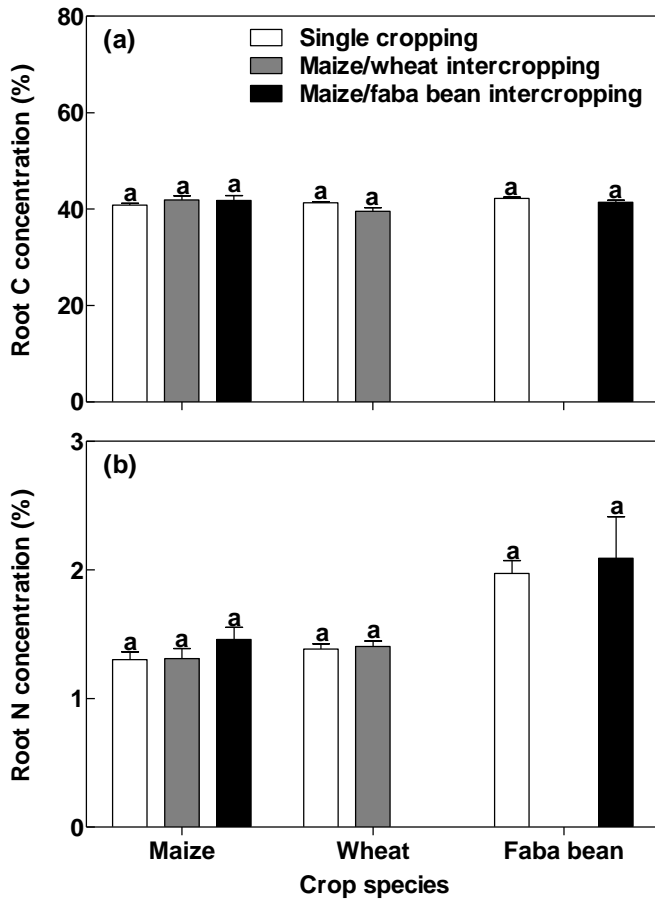
<sup>\*</sup>Aboveground biomass and <sup>†</sup>grain yield in 2008 has been published previously in Li et al., (2011c).



**Figure S4.1** Schedules of fertilization, irrigation and root sampling for wheat, maize and faba bean. Shaded areas represent growing periods of crop species. Large arrows (F) indicate times of fertilizer application, and small arrows (I) indicate timing of irrigation. The moments of root sampling are indicated with crosses.



**Figure S4.2** Soil bulk density at 0-20 and 20-40 cm depth in single cropping and intercropping plots. Data are means  $\pm$  SEM,  $N = 3$ . Means with the same letter do not differ significantly ( $P = 0.05$ ).



**Figure S4.3** Root C (A) and N (B) concentration of maize, wheat and faba bean in maize/wheat and maize/faba bean intercropping and single cropping. Data are means  $\pm$  SEM,  $N = 4$ . Means with the same letter do not differ significantly between treatments within the same crop ( $P = 0.05$ ).



# Chapter 5

## **Crop diversification by intercropping affects decomposition of soil organic matter and root litter**

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Wopke van der Werf



### Abstract

With diversification of crop systems, e.g. through intercropping, the diversity, quality and quantity of organic matter sources as well as associated abiotic and biotic attributes of the soil ecosystem are changed. We hypothesised that these intercropping-induced changes could influence the decomposition of soil organic matter (SOM) as well as fresh root litter. A soil incubation experiment was conducted to determine whether SOM decomposition differed between soil from intercropping and monocropping plots. A standard substrate (compost) treatment was used to determine effects mediated by the soil ecosystem. We found that SOM decomposed faster in soils from maize/wheat and maize/faba bean intercrops than expected from their monocrops. The soil ecosystem did not affect the rate of decomposition of compost, therefore the accelerating effect of intercropping was attributed to changes in SOM quality. Experiments were performed in the laboratory and in the field to examine intercropping effects on fresh root litter decomposition through changes in root litter diversity and/or the soil ecosystem. The soil ecosystem effect was further examined by a standard substrate (filter paper). Intercropping effects on decomposition of root litter depended on incubation conditions and crop combination. In the laboratory, maize/wheat intercropping decreased root litter decomposition through a soil-mediated effect. In contrast, maize/faba bean intercropping accelerated root litter decomposition in field conditions via a weak but significant litter diversity effect. The rate of decomposition of filter paper was higher in soil from intercropping than expected from the single crop soils. We conclude that intercropping influences the decomposition of organic matter in soil through a combination of effects acting via the soil ecosystem and via litter quantity and diversity. Both increased and reduced rates of decomposition were found. The changes have consequences for carbon sequestration in soil and are therefore relevant for CO<sub>2</sub> mitigation.

## Introduction

Intercropping is an agricultural practice in which two or more crop species/varieties are grown at the same time in the same field. There is ample evidence that intercropping enhances aboveground biomass production and grain yields (Lithourgidis et al., 2011; Willey, 1979). This higher production is related to increased resource acquisition resulting from niche complementarity and facilitation between intercropped species (Hinsinger et al., 2011), and reduced negative impacts of weeds, pests and diseases (Liebman and Dyck, 1993; Trenbath, 1993; Zhu et al., 2000). Recent studies have suggested that the greater aboveground biomass production is mirrored belowground (Ghosh et al., 2006; Li et al., 2011b; Yang et al., 2010). This could result in enhanced soil C sequestration, provided that decomposition of soil organic matter (SOM) and fresh root litter are unaffected by intercropping. However, our own observations indicate that intercropping greatly enhances belowground biomass input, but affects soil C storage in soil to a lesser extent (Cong et al., in prep.). A potential explanation for this discrepancy would be that intercropping increases decomposition. Here, we therefore address the question whether intercropping affects the rate of decomposition of organic matter in the soil.

Intercropping could affect organic matter decomposition in soil either via changes in the diversity, quality and quantity of organic matter, or through changes in the soil ecosystem. So far, one study compared decomposition rate of SOM in monocropping and intercropping soils (Rusinamhodzi et al., 2009), finding no significant differences. However, the intercropping history of the soil prior to measurements was only 2 years in this study and possibly not long enough to cause an intercropping effect on SOM decomposition.

**Litter diversity effect.** To assess effects of litter diversity, the rate of decomposition in a diversified system should be compared to the average rate from systems that consist of the same species in isolation (Wardle et al., 1997), keeping the amount of litter the same. High litter diversity can accelerate or inhibit decomposition compared to the average

decomposition of the single litters (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). Acceleration is often observed in litter mixtures consisting of high-N and low-N litters (Wardle et al., 1997) because N transfer from a high-N litter to a low-N litter could relieve N limitation in the decomposer community and thus enhance the rate of decomposition of low-N litter (Seastedt, 1984). This mechanism has been invoked to explain enhanced decomposition of low-N maize residues when mixed with high-N soybean residue in intercrops (Vachon and Oelbermann, 2011). On the other hand, litter decomposition can be inhibited if one of the components releases inhibitory compounds (e.g. polyphenols) impeding decomposition of both litters (Schimel et al., 1998). Since residues of annual crops in agroecosystems are usually low in inhibitory compounds (Cadisch and Giller, 1997), inhibition probably plays a minor role in intercrops.

**Litter quantity effect.** Dijkstra et al. (2005) found in a 4-year grassland experiment that the relative decomposition rate of SOM was higher in four-species plots than in monocultures. The result was largely attributed to greater soil C inputs resulting from increased biomass production in more diverse plots. Greater fresh biomass input can increase the relative proportion of labile C in total soil C, thus enhancing SOM decomposition. This mechanism might play a role in intercropping, because intercrops have greater aboveground biomass production (Lithourgidis et al., 2011; Willey, 1979) as well as greater C input into the soil through root residues than monoculture (Cong et al., in prep; Ghosh et al., 2006; Li et al., 2006, 2011b; Yang et al., 2010).

**Soil ecosystem effect.** Another potential mechanism is through alteration of the decomposition microenvironment (i.e. through changes in abiotic and biotic attributes of soil ecosystem). Studies in grassland systems have shown that the soil ecosystem affects decomposition of standard litter (Hector et al., 2000; Knops et al., 2001; Scherer-Lorenzen, 2008; Spehn et al., 2005; Vogel et al., 2013): Faster decomposition was found in soils from diverse species plots compared to monoculture plots. Vogel et al. (2013) attributed the faster decomposition to increased soil water availability in diverse species plots, while other studies suggest that plant diversity may enhance litter decomposition



through enhanced microbial biomass, greater microbial activity or greater enzymatic diversity in soil microbial communities (Zak et al., 2003; Stephan et al., 2000; Chung et al., 2007). There are no published reports on the effect of intercropping on fresh root litter decomposition via the soil ecosystem effect.

Here, we test the hypothesis that intercropping enhances the decomposition of SOM and root litter compared to monocropping. Soil samples were taken from a long term (7 years) intercropping experiment. We incubated them with and without a standard substrate to investigate (1) if intercropping influences SOM decomposition and (2) if the effect can be attributed to a soil ecosystem effect. We further performed experiments using a factorial design with soil and root litter as two factors under both laboratory and field conditions to unravel if and how intercropping affects root litter decomposition: through (1) litter diversity effect and/or (2) soil ecosystem effect. Two intercropping combinations (maize/wheat and maize/faba bean) were chosen to explore whether intercropping effects depend on the presence of legumes.

## Materials and methods

### **Field sampling**

A field experiment was initiated in 2003 in Wuwei, Gansu, China to study long-term effects of intercropping and rotation on productivity and soil properties. The experiment comprises thirteen crop systems based on maize, wheat and faba bean. Treatments are intercropping or monocropping, and rotation or continuous cultivation. It is laid out as a completely randomized block design with three blocks (Li et al., 2007). To exclude possible interference from rotation, we selected five continuous cropping systems among the thirteen treatments. They are (1) maize (*Zea mays* L.) continuous monocropping (hereafter referred to “maize”), (2) wheat (*Triticum aestivum* L.) continuous monocropping (“wheat”), (3) faba bean (*Vicia faba* L.) continuous monocropping (“faba bean”), (4) maize/wheat continuous intercropping (“maize/wheat”) and (5) maize/faba bean continuous intercropping (“maize/faba bean”). The maize/wheat and maize/faba bean

intercropping combinations were chosen because they are commonly practiced in Northwest China and they contrast a cereal/cereal and a cereal/legume intercrop. Maize was grown at a row distance of 40 cm, wheat at 13.3 cm and faba bean at 20 cm. The maize/wheat intercrop was planted in 1.6 m-wide strips that consisted of an 80 cm maize strip (2 rows) and an 80 cm wheat strip (6 rows). The maize/faba bean intercrop was planted in 1.6 m-wide strips that consisted of two rows maize and four rows faba bean. The intercrops thus had a relative density of both species of 50% as compared to the sole crops. The intercrops were cultivated yearly with each of the two crops growing on exactly the same place. Wheat and faba bean were sown in late March and harvested early July (wheat) or late July (faba bean). Maize was sown in mid-April and harvested early October. Wheat and faba bean received flood irrigation five times during the growing season, while the other treatments (all including maize) were irrigated two more times after harvest of faba bean to meet water demand of maize.

Soil samples (0-20 cm depth) were taken on 1 July 2010. Twenty cores per plot were taken in the middle between two rows in monocropping treatments. For intercropping, we took twenty cores in the middle between the rows of the two crop species. Soil samples were bulked per plot, transported to the lab, sieved (2 mm) to remove visible fresh plant material and macrofauna, and stored at 4° C until further processing. A subsample was air-dried for 3 days and treated with HCl to remove inorganic C (Midwood and Button, 1998). The acid-treated soil samples were ground in a ball mill prior to analysis. Fresh roots (< 2 mm diameter) of wheat, maize and faba bean were collected in June 2011 in monocropping plots, washed to remove soil particles, oven-dried at 70 °C for 48 h, and cut into pieces of approximately 1 cm length. C and N content of soil and root samples were determined prior to incubation by a vario MACRO Elemental Analyser (Elementar Company, Germany).

### ***Decomposition experiments***

We conducted three experiments to examine intercropping effects on decomposition of SOM and root litter. First, we conducted a laboratory experiment in which we monitored

CO<sub>2</sub> production from five types of soil samples incubated with/without a standard material (Experiment 1) to examine if intercropping affects SOM decomposition and establish whether an effect, if present, can be attributed to changes in the soil ecosystem or to the quality of SOM, or both. Second, we conducted another laboratory experiment using a two-way factorial design with “soil” and “root litter” as factors (Experiment 2). This factorial experiment confronted two monocropping soils and an intercropping soil to two single litters and a root mixture in all nine combinations. Using this design we could separate effects of soil ecosystem from those of root litter diversity, and establish whether or not those effects interact. The same factorial design was carried out in situ in the field in Gansu, China, to determine root litter decomposition by monitoring root mass loss in litterbags (Experiment 3).

#### ***Experiment 1: Decomposition of SOM and compost in the laboratory***

A standard amount of moist soil (50 g on dry weight basis) was mixed with and without 0.5 g oven-dried (70°C) compost. Compost was chosen as substrate because it is a recalcitrant material with a decomposition rate that is similar to that of SOM. Soils were subsequently placed in 250 mL-bottles that were closed with a gas-permeable cotton plug and incubated in the laboratory at 20 °C. Soil moisture content was kept at 60% of water holding capacity by supplying deionized water every 2 weeks. The production of CO<sub>2</sub> was measured four times: at 2, 4, 8 and 16 weeks after filling the bottles. Prior to these measurements, the bottles were flushed with compressed air for 15 minutes and closed with an air-tight lid and then incubated in the dark at 20 °C for 4 h. The increase in CO<sub>2</sub> concentration after this 4 h incubation was monitored using a photo-acoustic gas monitor (INNOVA 1412, AirTech Instruments, Ballerup, Denmark). Compost-derived CO<sub>2</sub> production was calculated by subtraction of the CO<sub>2</sub> efflux from soil alone from the total CO<sub>2</sub> efflux produced by compost-amended soil, assuming additivity.

### *Experiment 2: Decomposition of root litter and filter paper in the laboratory*

Two factorial experiments were conducted in the laboratory (Table 5.1): one for the maize/wheat intercropping and their monocropping systems (Experiment 2A) and one for the maize/faba bean intercropping and monocropping systems (Experiment 2B). Each trial combined three soils (monocrop 1, monocrop 2, and intercrop) with three root litters: from monocrop 1, from monocrop 2, and from intercrop (Table 5.1). The latter was a mixture of roots from the two monocrops. Because one treatment (maize root  $\times$  maize soil) was shared by the two experiments 2A and 2B, there were 17 treatments in total.

A standard amount of moist soil (50 g on dry weight basis) was mixed with 0.5 g oven-dried (70°C) root litter. The amount of root litter added to soil ensured sufficient amount of C for microbial respiration during incubation. Mixtures contained equal amounts of component litters. The incubation and monitoring procedures were the same as described for Experiment 1. Like compost, root-derived CO<sub>2</sub> production was calculated by subtraction of the CO<sub>2</sub> efflux from soil alone (Experiment 1) from the total CO<sub>2</sub> efflux produced by root-amended soil.

### *Experiment 3: Decomposition of root litter and filter paper in the field*

The experimental design of Experiment 2 (Table 5.1) was applied in a litter bag experiment conducted in the field in Wuwei, Gansu, China in 2011. In addition to root litters, cellulose filter paper (N content of 0.03%) was used as substrate to test the soil ecosystem effect on decomposition. Bags (5  $\times$  5 cm; 250  $\mu$ m nylon mesh) were filled with 1 g (dry weight basis) of oven-dried root samples or filter paper. The root mixtures contained equal amounts of component root litters. A total of 396 litterbags (seventeen root  $\times$  soil combinations plus five filter paper  $\times$  soil combinations, two litter bags for each combination, three field blocks and three harvests:  $(17+5) \times 2 \times 3 \times 3 = 396$ ) were buried at 10 cm soil depth on 15 June 2011. The bags were placed in the middle between two rows in monocropping treatments, or in the middle between rows of different crop

**Table 5.1** Factorial design of two experiments (lab and field), with soil and root litter as two factors for two intercropping systems and their corresponding monocropping systems***Maize/wheat intercropping and monocropping systems (Experiment 2A)***

Soil	Root litter		
	Maize	Wheat	Maize/Wheat
Maize	<u>x</u> <sup>a,b</sup>	x	x
Wheat	x	<u>x</u>	x
Maize/wheat	x	x	<u>x</u>

***Maize/faba bean intercropping and monocropping systems (Experiment 2B)***

Soil	Root litter		
	Maize	Faba bean	Maize/faba bean
Maize	<u>x</u>	x	x
Faba bean	x	<u>x</u>	x
Maize/faba bean	x	x	<u>x</u>

<sup>a</sup>Underlined crosses indicate root litters decomposed in their own soils.

<sup>b</sup>The treatment of maize root litter x maize soil is shared by the two factorial designs.

species in intercrops. Litter bags were dug up at exactly 2, 6, and 12 months after burial, and oven-dried (70 °C, 48 h). Decomposing root litter or filter paper was gently retrieved by carefully brushing away adhering soil particles and other extraneous material, and then weighed.

***Quantitative comparison of decomposition rates***

To quantitatively describe the decomposition process, total integrated decomposition over time, and compare treatment effects, we fitted mathematical models to measurements of CO<sub>2</sub> efflux in laboratory trials. Two equations were tested. The first one is a first-order exponential decay:

$$Y_t = Y_0 \exp(-kt) \quad (\text{Eq. 1})$$

where  $Y_t$  and  $Y_0$  are the amount of organic C or root mass present at time  $t$  and time 0, respectively, and  $k$  is the constant relative decomposition rate.

The second model has a time-dependent relative decomposition rate (Yang and Janssen, 2000), accounting for a slowing down of the decomposition rate over time as the easily decomposable components disappear as CO<sub>2</sub>, resulting in a greater proportion of recalcitrant components in the remaining substrate. The average relative decomposition rate ( $K_t$ ) between time = 0 and time =  $t$  decreases over time according to a log-log relationship (Yang and Janssen, 2000):

$$\log(K_t) = \log(K_1) - S \log(t) \quad (\text{Eq. 2a})$$

$$K_t = K_1 t^{-S} \quad (\text{Eq. 2b})$$

where  $K_1$  is the relative decomposition rate in the first time step and  $S$  is a measure of the speed at which  $K_t$  decreases over time. Greater values of  $S$  signal faster decline in decomposition rate.

Substitution of  $k$  in Eq. 1 by Eq. 2b yields:

$$Y_t = Y_0 \exp(-K_1 t^{1-S}) \quad (\text{Eq. 3})$$

The CO<sub>2</sub>-C production at any time  $t$  is given by the first derivatives of Eq. 1 and Eq. 3:

$$dY_t/dt = -kY_0 \exp(-kt) \quad (\text{Eq. 4})$$

$$dY_t/dt = [-K_1(1-S)t^{-S}]Y_0 \exp(-K_1 t^{1-S}) \quad (\text{Eq. 5})$$

Measured CO<sub>2</sub> production over the time of incubation (4 hours) was converted to daily quantity,  $\Delta\text{CO}_2\text{-C}$ , by multiplication with six and molecular mass ratio of 12/44. Equations 4 and 5 were fitted to  $\Delta\text{CO}_2\text{-C}$ , solving for  $k$  (Eq. 4) or  $K_1$  and  $S$  (Eq. 5), using non-linear regression (SPSS 20.0). Ordinary sums of squares was used as goodness of fit criterion. Fitting was done for each individual block in the field from which the soil originated, resulting in a mean and standard error of model results. The calibrated parameters  $k$ ,  $K_1$  and  $S$  were used to calculate the cumulative amount of C decomposed ( $CCD$ , g CO<sub>2</sub>-C kg<sup>-1</sup> soil) of the sample over 112 days of incubation. If neither Eq. 4 nor Eq. 5 described the data satisfactorily, we used linear interpolation between subsequent measurements to calculate the  $CCD$  (Dorette et al., 2011). The  $CCD$  was expressed as

percentage of C decomposed ( $CD$ ), taking into account the initial amount of C in the sample:

$$CD (\%) = CCD/C_0 * 100 \quad (\text{Eq. 6})$$

where  $C_0$  is the amount of C from the sample at  $t = 0$  (g C kg<sup>-1</sup> soil).

The expected  $CD$  (%) from the sample from an intercrop of species A and B was calculated on the basis of the weighted organic C of the sample from two monocrops as follows:

$$CD_{exp}(\%) = CD_A(\%) \frac{C_A}{C_A + C_B} + CD_B(\%) \frac{C_B}{C_A + C_B} \quad (\text{Eq. 7})$$

where  $CD_A$  (%) and  $CD_B$  (%) are the percentage of C decomposed from the sample from monocrops species A and species B, respectively.  $C_A$  and  $C_B$  are initial organic C of the sample (g C kg<sup>-1</sup> soil) of monocrops species A and species B, respectively.

The expected root mass loss (%) in a root mixture of species A and B was calculated as the average of root mass loss (%) of monocrop species A and B because the mixture consisted of equal amount of component root litters. Expected amounts were calculated separately for each block to enable statistical analysis.

### **Statistical analyses**

Repeated-measures ANOVA was done to analyse the effect of intercropping on the time course of (1) CO<sub>2</sub> production derived from SOM and root litters in the laboratory and (2) mass loss (%) of root litters and filter paper in the field. One-way analysis of variance was used to analyse C, N content and C/N ratio of soils and roots. Two-way analysis of variance was used to evaluate the main and interactive effects of substrate quality and soil ecosystem on decomposition of SOM and substrates. Once the main effect was found significant, Tukey's post hoc test was used for pair-wise comparisons. And we further tested whether the rate of decomposition of mixed litter is different from the average of that of the single litters (i.e. litter diversity effect). All data met the ANOVA assumptions of normality and equal variance.

**Table 5.2** Carbon and nitrogen content and C/N ratio of soils ( $n = 3$ ) originating from five cropping systems and of roots ( $n = 4$ ) of three crop species from monocropping systems (means  $\pm$  SEM)

Cropping system		C	N	C/N
		%	%	-
Soil	Maize	1.24 $\pm$ 0.03 <sup>a</sup>	0.14 $\pm$ 0.07 <sup>a</sup>	8.75 $\pm$ 0.47 <sup>a</sup>
	Wheat	1.20 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.04 <sup>a</sup>	7.66 $\pm$ 0.22 <sup>a</sup>
	Faba bean	1.21 $\pm$ 0.01 <sup>a</sup>	0.15 $\pm$ 0.05 <sup>a</sup>	7.92 $\pm$ 0.33 <sup>a</sup>
	Maize/wheat	1.24 $\pm$ 0.02 <sup>a</sup>	0.16 $\pm$ 0.02 <sup>a</sup>	7.74 $\pm$ 0.09 <sup>a</sup>
	Maize/faba bean	1.25 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.03 <sup>a</sup>	7.86 $\pm$ 0.09 <sup>a</sup>
Root	Maize	40.8 $\pm$ 0.4 <sup>b</sup>	1.30 $\pm$ 0.06 <sup>b</sup>	31.6 $\pm$ 1.7 <sup>a</sup>
	Wheat	41.3 $\pm$ 0.3 <sup>b</sup>	1.38 $\pm$ 0.04 <sup>b</sup>	29.9 $\pm$ 0.9 <sup>a</sup>
	Faba bean	42.2 $\pm$ 0.3 <sup>a</sup>	1.97 $\pm$ 0.10 <sup>a</sup>	21.6 $\pm$ 1.3 <sup>b</sup>

Means with the same letters are not significantly different in a Tukey's *post hoc* test ( $P < 0.05$ ).

## Results

### ***C and N content and C/N ratio of soil and root samples***

The C and N content and C/N ratio were similar among the soil samples of the intercropping and monocropping plots (Table 5.2). When comparing the expected soil C and N content for both maize/wheat and maize/faba bean intercropping systems with the observed ones, we found that intercropping slightly but not significantly increased soil C content by 2% ( $P = 0.16$ ), and significantly increased soil N content by 8% ( $P = 0.008$ ) compared to expected from monocropping. Both effects were independent of intercropping combination ( $P = 0.83$  for soil C;  $P = 0.87$  for soil N for interaction effects).

The C and N content and C/N ratio were significantly different among root samples (Table 5.2). Maize and wheat roots had similar C and N contents, but C content was somewhat higher and N content substantially higher in faba bean root than in wheat or maize roots. The C/N ratio of faba bean root was lower than of maize or wheat roots.

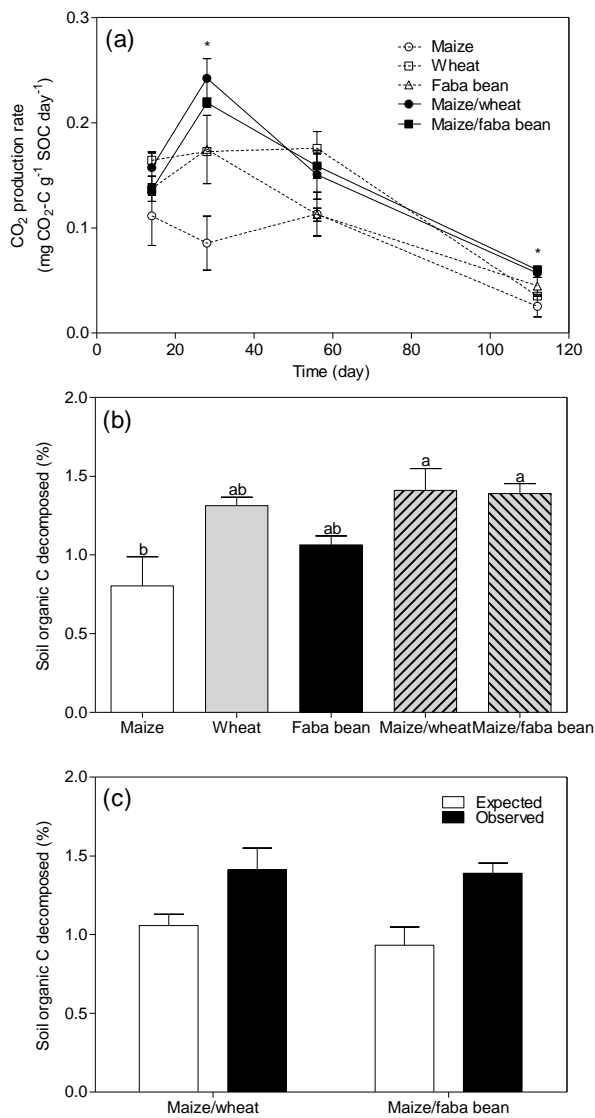


### **Experiment 1: Decomposition of SOM and compost in the laboratory**

The CO<sub>2</sub> production from unamended soil samples tended to first increase, then decrease during incubation under laboratory conditions (Fig. 5.1a). There were significant differences in the time courses among the soils from the five cropping systems (Cropping system  $\times$  time:  $P = 0.01$ ): The CO<sub>2</sub> production was significantly higher from soils originating from the two intercropping treatments than from maize monocropping after 28 and 112 days of incubation but no significant treatment effects were observed after 14 and 56 days.

Neither of the two decomposition models could be calibrated to describe the observed CO<sub>2</sub> production satisfactorily: The  $r^2$  values were  $0.02 \pm 0.01$  (mean  $\pm$  SD,  $n = 15$ ) for the exponential decay model and  $0.39 \pm 0.18$  for the Yang and Janssen model. Using linear interpolation between observed CO<sub>2</sub> fluxes at different times, we calculated the percentage of C decomposed over the incubation period ( $CD$ ) (Eq. 6) to compare treatment effects. We found that  $CD$  was significantly higher in soil samples from maize/wheat and maize/faba bean intercropping plots than from maize monocropping plots, and the samples from wheat and faba bean monocropping plots were intermediate ( $P = 0.03$ ; Fig. 5.1b).

We calculated the expected  $CD$  for the intercropping samples based on the CO<sub>2</sub> production measured in the samples from monocropping treatments. Regardless of crop combination (Intercropping  $\times$  combination:  $P = 0.65$ ), intercropping significantly increased SOM decomposition ( $P = 0.01$ ; Fig. 5.1c): The observed C decomposed (%) was 1.3 times the expected in maize/wheat intercropping and 1.5 times the expected in maize/faba bean intercropping.



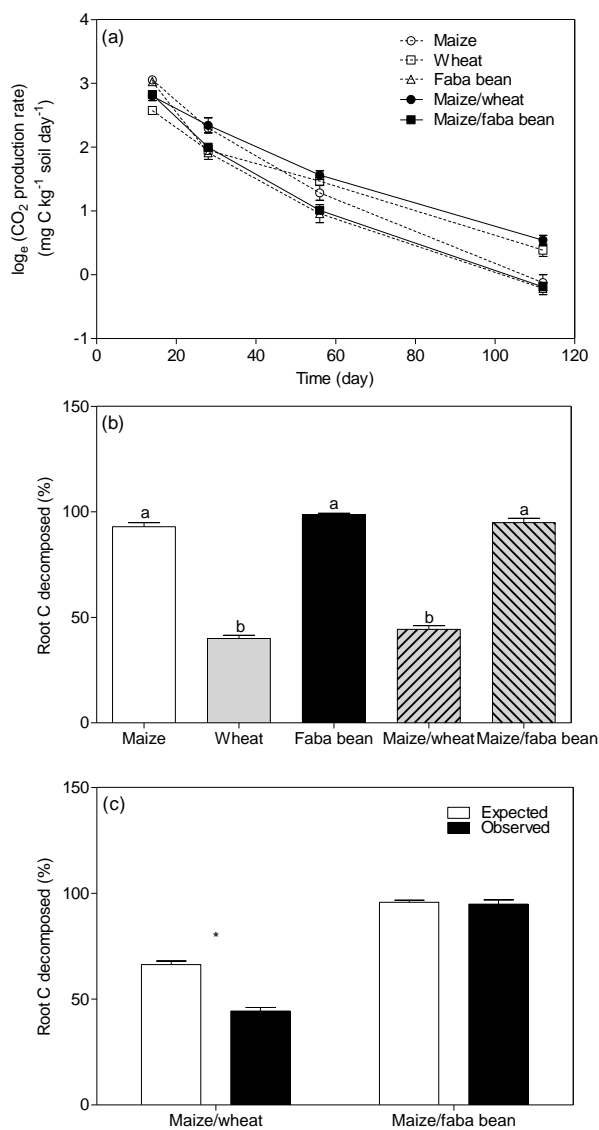
**Figure 5.1** Intercropping effect on soil organic matter decomposition in the laboratory. (a) Time course of CO<sub>2</sub> production from unamended soils from five cropping systems, (b) soil organic C decomposed (%) between day 14 and day 112 and (c) expected and observed soil organic C decomposed (%) for two intercropping systems. Expected values were calculated from observations in monocropping systems. Data are means  $\pm$  SEM,  $n = 3$ . Asterisks in (a) refer to significant differences between treatments ( $P < 0.05$ ). Means with the same letter in (b) are not significantly different in a Tukey's *post hoc* test ( $P < 0.05$ ).

To examine whether intercropping affects SOM decomposition through alternations of the soil ecosystem, we added compost to the same soil samples as above. The total CO<sub>2</sub> production significantly increased by 23% due to addition of compost ( $P < 0.01$ ), independent of the origin of the soil (Cropping system  $\times$  compost:  $P = 0.92$ ). The compost-derived component of CO<sub>2</sub> production was similar across the soils from the five cropping systems ( $P = 0.35$ ). The absence of a soil ecosystem effect on compost decomposition indicates that intercropping did not affect the ability of soil ecosystem to decompose SOM.

### ***Experiment 2: Decomposition of root litter and filter paper in the laboratory***

We first explored the intercropping-induced combined effects of litter diversity and soil ecosystem on root litter decomposition by analysing treatments with root litters added to their “own” soils (Table 5.1). The root-derived component of the CO<sub>2</sub> production significantly decreased over time for all five root litters ( $P < 0.01$ ; Fig. 5.2a), but there were significant differences in the time courses among them (Root litter  $\times$  time:  $P < 0.01$ ). The slopes of the logarithmic CO<sub>2</sub> production from root litters of wheat and maize/wheat mixture against time were smaller than that of other three root litters, indicating a lower relative decomposition rate of these two litters.

The two decomposition models were fitted to describe the observed root-derived CO<sub>2</sub> production. The model by Yang and Janssen (2000) fitted all data well, with an average  $r^2$  of  $0.99 \pm 0.01$ . The exponential decay model did not describe the observations satisfactorily ( $r^2 = 0.48 \pm 0.23$ ). Thus, the Yang and Janssen model with calibrated parameters  $K_1$  and  $S$  was used to calculate root C decomposed (%) of five root litters (Fig. 5.2b). Root C decomposed (%) was significantly higher from roots of maize, faba bean and maize/faba bean mixture than that of wheat and maize/wheat root mixture in their respective own soils ( $P < 0.01$ ).

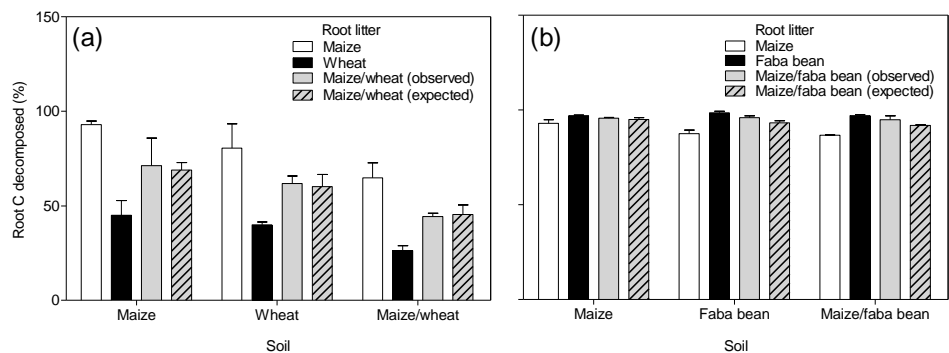


**Figure 5.2** Intercropping effects on root litter decomposition in the laboratory. (a) Time course of root-derived CO<sub>2</sub> production in their own soils from five cropping systems, (b) root C decomposed (%) over 112 days of incubation and (c) expected and observed root C decomposed (%) from two intercropping systems. Expected values were calculated from the observations in monocropping systems. Data are means  $\pm$  SEM,  $n = 3$ . Means with the same letter in (b) are not significantly different in a Tukey's *post hoc* test ( $P < 0.05$ ). Asterisks in (c) refer to significant differences between treatments ( $P < 0.05$ ).

Expected root C decomposed (%) for the maize/wheat and maize/faba bean intercropping treatments was calculated based on root-derived CO<sub>2</sub> production measured in the monocropping treatments. We found that intercropping differentially affected root litter decomposition, depending on crop combination ( $P < 0.01$ ; Fig. 5.2c). Observed root C decomposed (%) was significantly lower (-33%) than expected in maize/wheat intercropping ( $P < 0.01$ ), whereas no difference was found between observed and expected root C decomposed (%) in maize/faba bean intercropping ( $P = 0.65$ ). This indicates that maize/wheat intercropping retarded root decomposition, while maize/faba bean intercropping did not affect it under laboratory conditions.

We further used all treatments of experiments 2A and 2B (Table 5.1) to separate the combined effect of intercropping on root litter decomposition into two potential pathways: through a litter diversity effect and through a soil ecosystem effect (Fig. 5.3). Root litters significantly affected decomposition in mono- and inter-cropping systems with maize and wheat ( $P < 0.01$ , Fig. 5.3a): Maize roots decomposed faster than wheat roots, and the root mixture of maize and wheat decomposed at an intermediate rate, independent of the soils with which they were mixed (Litter  $\times$  soil ecosystem:  $P = 0.97$ ). The maize/wheat root mixture decomposed as expected from the decomposition of the single litters ( $P = 0.89$ ), indicating that root litter diversity did not affect the decomposition rate. Root decomposition significantly varied with soil ecosystem ( $P = 0.004$ ). Across root litters, decomposition was fastest in soils from maize and wheat monocropping plots and slowest in soils from maize/wheat intercropping plots ( $P = 0.002$ ). This indicates that the soil ecosystem from maize/wheat intercropping plots slowed down root decomposition.

In maize/faba bean monocropping and intercropping systems, 85-95% of initial root C was decomposed in all root samples after 112 days of incubation (Fig. 5.3b). The rate of decomposition significantly differed among root litters ( $P < 0.01$ ), but the effect varied with soil ecosystem (Root litter  $\times$  soil ecosystem:  $P = 0.03$ ). Maize roots decomposed more slowly than faba bean roots and maize/faba bean root mixture, only when they



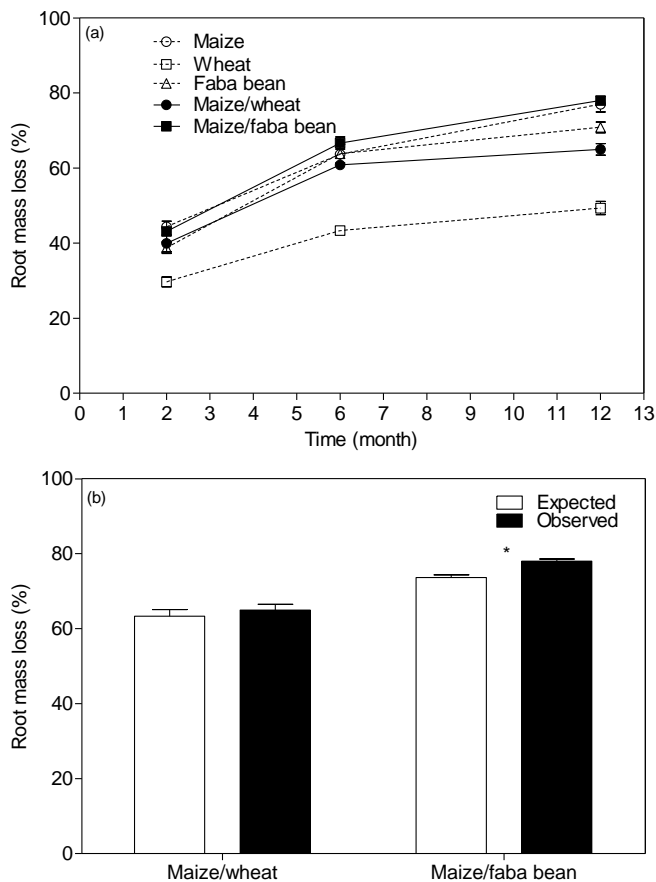
**Figure 5.3** Effects of litter diversity and soil ecosystem on decomposition of root litter in the laboratory. Root C decomposed (%) of maize, wheat and maize/wheat mixture (a) and of maize, faba bean and maize/faba bean mixture (b) mixed with various soils after 112 days of laboratory incubation. Expected root C decomposed (%) of root litter mixtures is calculated from root litters of single species in the same soil. Data are means  $\pm$  SEM,  $n = 3$ .

were mixed with faba bean soil and maize/faba bean soil. Soil ecosystem affected the decomposition of root litter at a marginal significance level ( $P = 0.06$ ). Specifically, Maize roots decayed slightly faster (7%) in their own soils than in the other two soils ( $P = 0.03$ ). We further tested the litter diversity effect: the observed decomposition rate of the mixture of maize and faba bean roots was slightly (2%) but significantly ( $P = 0.02$ ) higher than expected from single root litters of maize and faba bean. This accelerating effect of the mixture was independent of the soil ecosystem (Litter diversity  $\times$  soil ecosystem:  $P = 0.47$ ). Soil ecosystem affected the decomposition of root litter at a marginal significance level ( $P = 0.06$ ), but Tukey's post hoc test did not show significant differences between soil ecosystems. Similar results were obtained when evaluating the effects of litter diversity and soil ecosystem on root litter decomposition after 14, 28 and 56 days of incubation.

### ***Experiment 3: Decomposition of root litter and filter paper in the field***

As in experiment 2, we first explored the combined effects of intercropping on root litter decomposition using treatments with root samples in litterbags buried in their “own” soils (Table 5.1). Root mass loss (%) significantly increased over time for all five root litters ( $P < 0.01$ ; Fig. 5.4a). Wheat root litter decomposed significantly more slowly than others in their own soils throughout the whole field incubation period ( $P < 0.001$ ).

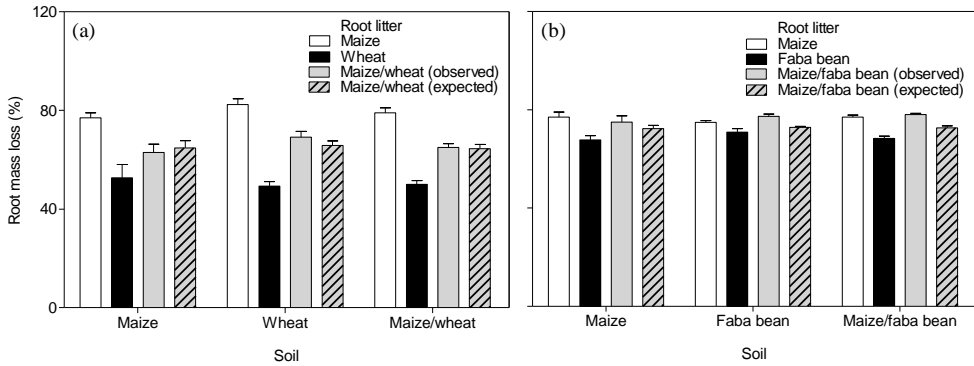
Expected root mass loss (%) in the maize/wheat and maize/faba bean intercropping treatments was calculated based on observed root mass loss (%) after 1 year in the monocropping treatments. In contrast to the findings under laboratory conditions, observed root mass loss (%) was significantly greater than expected values by 6% in maize/faba bean intercropping ( $P = 0.04$ ; Fig. 5.4b), while no difference was found between observed and expected root mass loss (%) in maize/wheat intercropping system ( $P = 0.55$ ). This indicates that maize/faba bean intercropping increased root decomposition, while maize/wheat intercropping did not affect it under field conditions. The factorial design allowed distinguishing between the two pathways along which intercropping could affect root litter decomposition: through litter diversity and/or through soil ecosystem. In maize/wheat mono- and intercropping treatments, root decomposition varied significantly depending on root litter composition ( $P < 0.01$ ). Regardless of soil ecosystem, maize roots decomposed faster than wheat roots (Fig. 5.5a). Maize/wheat root mixture decomposed as expected from the decomposition of the single litters ( $P = 0.89$ ), indicating litter diversity did not affect root decomposition. Root decomposition did not vary with soil ecosystem ( $P = 0.37$ ).



**Figure 5.4** Intercropping effects on root litter decomposition in the field litterbag experiment. (a) Time course of mass loss (%) of roots in litterbags mixed with their own soils from five cropping systems and (b) expected and observed root mass loss (%) in soils from two intercropping systems after 1 year of field incubation. Data are means  $\pm$  SEM,  $n = 3$ . Several errors bars in (a) are too small to be visualized.

For maize/faba bean intercropping and monocropping systems, root mass loss (%) was greater in maize roots than in faba bean roots ( $P < 0.01$ ; Fig. 5.5b). And maize/faba bean root mixture decomposed faster (6%) than expected from single litters ( $P = 0.002$ ; Fig. 5.5b), independent of the soil ecosystem (Litter diversity  $\times$  soil ecosystem:  $P = 0.56$ ). This

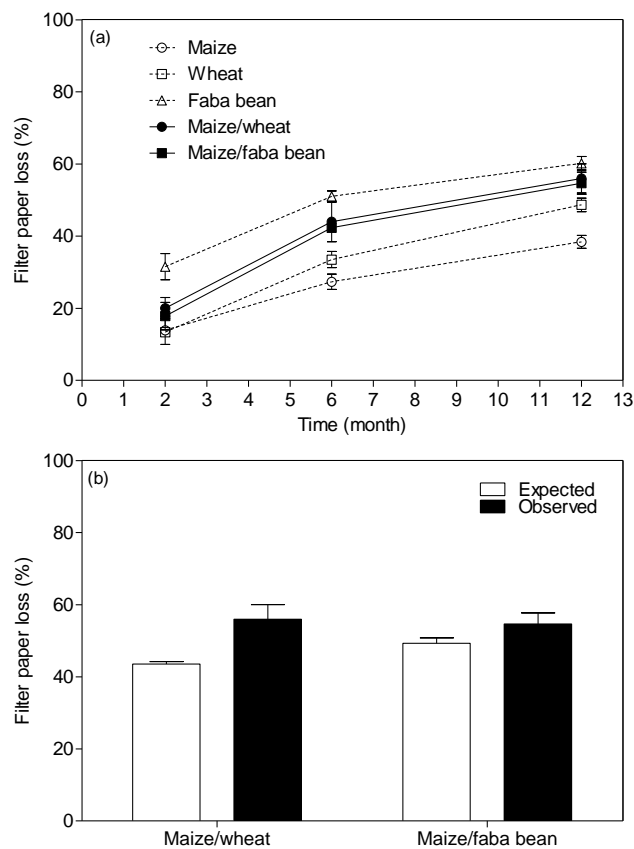




**Figure 5.5** Effects of litter diversity and soil ecosystem on decomposition of root litter in the field litterbag experiment. Root mass loss (%) of maize, wheat and maize/wheat mixture (a) and of maize, faba bean and maize/faba bean mixture (b) mixed with various soils after 1 year of field incubation. Expected root mass loss (%) of root litter mixtures is calculated from root litters of single species in the same soil. Data are means  $\pm$  SEM,  $n = 3$ .

suggested a positive litter diversity effect in root mixture of maize and faba bean. Again, we found that root decomposition did not differ in different soils ( $P = 0.53$ ).

We further used filter paper as a standard material to test the soil ecosystem effect on decomposition. Filter paper decomposed differently across the five soil ecosystems ( $P = 0.005$ ; Fig. 5.6a). It decomposed slowest in soils from maize monocropping plots, fastest in soils from faba bean monocropping, and intermediate in other soils. The expected mass loss (%) of filter paper was significantly higher in intercropping systems than expected from the average of monocropping systems ( $P = 0.02$ ; Fig. 5.6b), irrespective of intercropping combination (Intercropping  $\times$  combination:  $P = 0.26$ ).



**Figure 5.6** Effect of soil ecosystem on decomposition of filter paper in the field litterbag experiment. (a) Time course of mass loss (%) of filter paper in litterbags mixed with soils from five cropping systems and (b) expected and observed mass loss (%) of filter paper in soils from two intercropping systems after 1 year of field incubation. Data are means  $\pm$  SEM,  $n = 3$ .

## Discussion

### ***Intercropping effect on SOM and compost decomposition***

To our knowledge, this is the first report on an effect of intercropping on soil organic matter dynamics. Intercropping enhanced the relative decomposition rate of SOM compared to monocropping in both maize/wheat and maize/faba bean combinations (Fig. 5.1). These findings explain why intercropping insignificantly (Table 5.2) or only

slightly increased soil C content (Cong et al., in prep.). The enhanced decomposition rate will affect how much C sequestration can result from the substantially greater root biomass input in intercropping systems. Intercropping can affect SOM decomposition through changes in SOM quality and/or changes in the soil ecosystem. Our results showed that compost decomposed at a similar rate when it was added as a standard material to soils from five cropping systems, indicating that intercropping enhanced decomposition of SOM through changes in the quality of SOM. The change in SOM quality may partly be related to more C allocation into the soil (Cong et al., 2014; Ghosh et al., 2006; Li et al., 2011b; Yang et al., 2010) as a consequence of intercropping-induced greater aboveground biomass production (Li et al., 2007). This could have led to a faster rejuvenation of the SOM pool with more labile SOM (since the start of the experiment, 7 years ago), resulting in a higher relative decomposition rate. This “quantitative” effect of intercropping on C cycling, but not on storage (Table 5.2) was found in both maize/faba bean and maize/wheat combinations (Fig. 5.1c). This extends the accelerating effect of species diversity on long-term soil organic C cycling found in natural grasslands with legumes (Dijkstra et al., 2005; Reid et al., 2012) to agroecosystems without legumes. Obviously, additional N input through N<sub>2</sub> fixation by legumes is not a prerequisite for this accelerating effect of higher species diversity.

### ***Intercropping effect on fresh root litter decomposition***

The effect of intercropping on root litter decomposition was not straightforward, but depended on the crop combination and the incubation conditions. Maize/faba bean intercropping slightly increased root litter decomposition compared to expected from monocrops under field conditions (Fig. 5.4b). The overall effect of intercropping was attributed to accelerating decomposition in maize/faba bean root mixture (i.e. a positive litter diversity effect) (Fig. 5.3b, 5.5b), though the effect size was minor (2% for Fig. 5.3b; 6% for Fig. 5.5b). This result confirms the finding of Vachon and Oelbermann (2011) that the decomposition of maize crop residues was enhanced by mixing with legume (soybean) residue. We did not find a litter diversity effect of mixing roots of maize and wheat (Fig. 5.3a, 5.5a). While we compared only one intercropping system with a legume

with only one without a legume, our results fit in a general picture that litter diversity effects occur mainly when the two component species differ in resource quality, such as maize and faba bean root litters which differ in C/N ratio (Table 5.2). This is in line with what has been observed in natural systems in which leaf litter rather than root litter is the main substrate for study (Hättenschwiler et al., 2005; Pérez-Harguindeguy et al., 2008; Vos et al., 2013; Wardle et al., 1997).

Maize/wheat intercropping substantially slowed down root litter decomposition compared to expected from monocrops in the laboratory (Fig. 5.2c). The overall effect of intercropping may be attributed to the changes in soil chemistry and/or in soil microbiology because soil physical conditions were identical during incubation. Intercropping can decrease the accumulation of soil nitrate in the soil profile compared to monocropping (Li et al., 2005), which may retard decomposition of low-N root litter. Recent studies have shown that intercropping can modify rhizosphere microbial biomass and community composition (He et al., 2013; Song et al., 2007; Wang et al., 2007), which could be another reason for reduced rate of decomposition of root litter.

Surprisingly, we did not find any soil ecosystem effect on root litter decomposition in maize/wheat intercropping in the field (Fig. 5.5a). The negative soil ecosystem effect observed in the laboratory may have been counteracted by a positive intercropping effect through changes in abiotic factors in field conditions. In grassland studies, greater decomposition of standard litter in diverse species plots than monoculture plots was attributed to more favourable environmental conditions, e.g. increased soil moisture content (Spehn et al., 2005; Vogel et al., 2013). In this study, wheat and faba bean were harvested earlier than maize and two additional irrigations were applied to meet maize water demand. Thus, under favourable moisture conditions, more N mineralized from root residues of earlier crops would be available in intercropping systems, consequently stimulating the turnover rate of low-N wheat litter within litter bags. This was confirmed by our finding that very low-N filter paper (N content of 0.03%) decomposed faster in intercropping plots than in monocropping plots.

## **Conclusions**

We conclude that intercropping accelerates SOM decomposition. As a consequence, the soil C sequestration potential of intercropping via enhanced biomass input is partly counteracted by faster C cycling. Legume-based intercropping may further weaken this potential via accelerated root litter decomposition, compared to systems without legumes.

## **Acknowledgements**

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# Chapter 6

## General discussion







In this final chapter, I first present the main findings which address the research objectives given in the introduction of this dissertation. Then, I discuss these findings in the context of both the effects and underlying mechanisms of plant species diversity on SOM dynamics by contrasting species-diverse grasslands with intercropping agroecosystems. Next, I assess the impact of plant species diversity on soil C sequestration potential in the two ecosystems and discuss the implications for long-term soil fertility. Finally, I propose future research directions aimed at furthering our understanding of the mechanisms underlying plant species diversity effects on long-term soil C and N dynamics, and comprehensively evaluating a broad suite of ecosystems services.

### ***Plant and crop species diversity effects on SOM dynamics***

The research presented in this thesis shows that soil C and N storage increased with plant species richness in an 11-year grassland biodiversity experiment without the presence of legumes (Chapter 2). Although this pattern has been shown in previous studies (Fornara and Tilman, 2008; Steinbeiss et al., 2008), these studies all included legumes. Legumes constitute an important N input in N-limited grasslands through biological N<sub>2</sub> fixation and have shown strong positive effects on productivity (HilleRisLambers et al., 2004; Marquard et al., 2009) and soil C and N accumulation (Fornara and Tilman, 2008). As a consequence, greater soil C and N storage in higher diversity mixtures may be caused by the increased probability of legumes being included in such mixtures. Hence, our results provide the first evidence that additional input of N by legumes is not a prerequisite for enhanced soil C and N storage. Greater soil C and N storage were mainly attributed to increased soil C input and N retention, resulting from enhanced productivity associated with increased plant diversity. We showed that plant diversity enhances SOC decomposition (Chapter 2), extending the accelerating effect on SOC decomposition found in grasslands with legumes (Dijkstra et al., 2005; Reid et al., 2012) to grasslands without legumes. Furthermore, plant diversity also accelerated decomposition of fresh root litter (single litter or mixture) via changes in the soil decomposition microenvironment, not via alterations of litter mixing (Chapter 3). These results extend on some of the findings reported in the literature regarding positive effects of species

richness on the decomposition of single leaf litters (Hector et al., 2000; Spehn et al., 2005; Vogel et al., 2013) to the decomposition of mixed root litters.

In intercropping agroecosystems, we document a heretofore unrecognized advantage of intercropping: enhanced storage of soil C and N (Chapter 4). Moreover, such increase is independent of the presence or absence of legumes in intercropping combinations. We also demonstrated that belowground overyielding in terms of peak root biomass occurs in intercropping systems. This confirms, for the first time, that aboveground overyielding, which has been extensively demonstrated (e.g. Vandermeer, 1989; Lithourgidis et al., 2011) can be mirrored belowground. Increased root biomass input provides a plausible mechanism for the observed greater soil C storage. However, the increase in root biomass input (23%) did not lead to a proportionate increase in soil C storage after 7 years (4%). One possible explanation for this is that decomposition of SOC and/or root litter was stimulated by intercropping. Indeed, our results demonstrated that intercropping increases the relative decomposition rate of SOC decomposition compared to monocropping (Chapter 5). This acceleration effect was attributed to the changes in SOM quality, as opposed to changes in decomposition microenvironment. A 2-year study showed no effect of intercropping on SOC decomposition (Rusinamhodzi et al., 2009), presumably because the duration of the experiment was too short to allow time for sufficient changes in SOM quality. These results suggest that long-term experiments are required to examine the responses of SOC dynamics to changes in plant diversity. In addition, we demonstrate that intercropping affects decomposition of fresh root litter through a combination of effects acting via changes in soil decomposition microenvironment and changes in litter mixing (Chapter 5).

### ***Patterns and mechanisms underlying SOM dynamics in perennial N-limited grasslands versus annual N-rich intercropping agroecosystems***

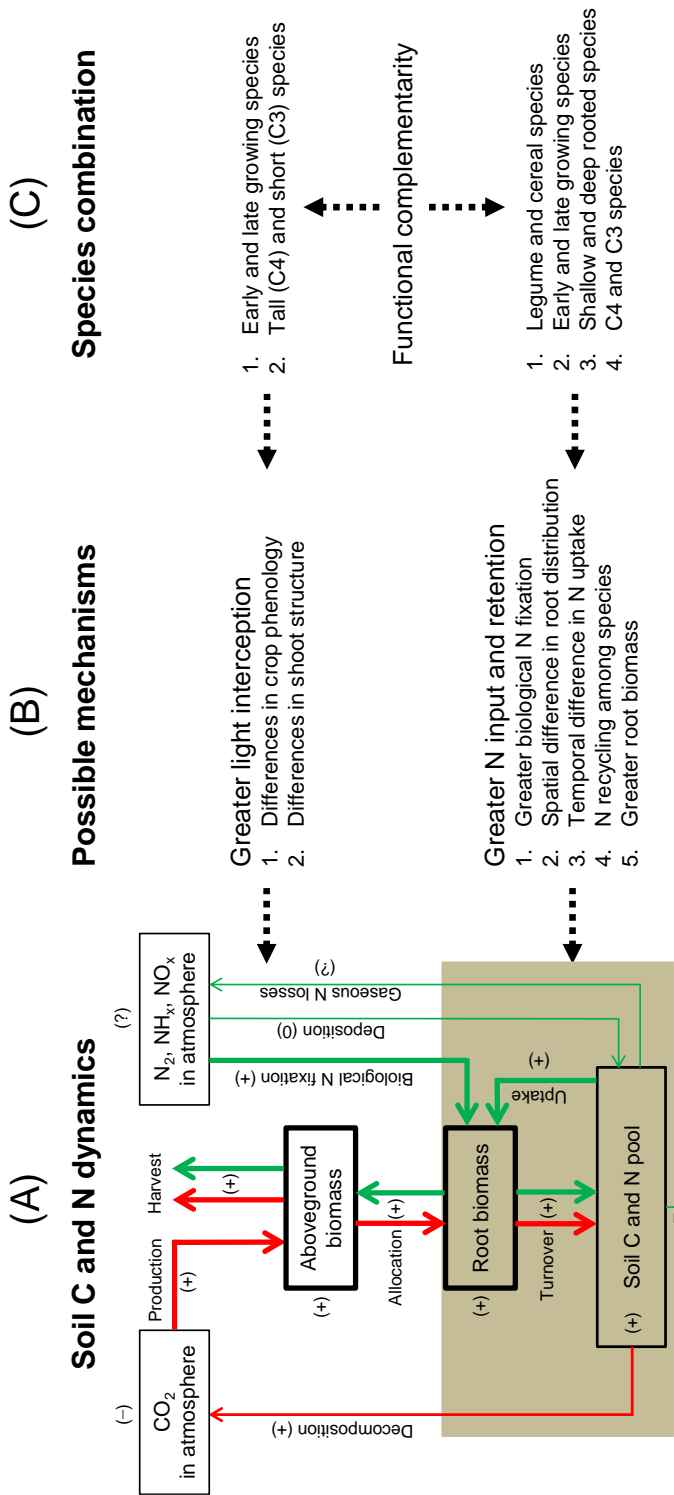
A general picture emerges from our results, in combination with the findings from the “Cedar Creek” and “Jena” grassland biodiversity experiments with legumes (Zak et al., 2003; Dijkstra et al., 2005; Fornara and Tilman, 2008; Steinbeiss et al., 2008; Marquard et

al., 2009; Reich et al., 2012). That is, the positive effects of plant species diversity on several ecosystem functions (i.e. soil C and N storage, biomass production and SOC decomposition) reported for perennial N-limited grasslands are paralleled in annual N-rich intercropping agroecosystems. The increase in soil C storage in both systems can be attributed to increased soil C input through increased biomass production, partly compensated by enhanced SOC decomposition. The increase in soil N storage is ascribed to increased soil N input in systems with legumes or increased N retention in systems without legumes (Fig. 1A).

Questions remain as to which mechanisms can explain the increase in soil C and N input or N retention. Studies have shown that species-diversified systems that consists of species with differing growing periods and/or canopy structure can intercept more light than monocultures, resulting in greater biomass (Anten and Hirose, 1999; Zhang et al., 2008) (Fig. 1B). This mechanism is likely to result in greater belowground C input as crop species maintain a functional balance between the shoot and the root system (Poorter et al., 2012). Greater soil N input can be attributed to enhanced biologically fixed N by legumes in cereal/legume intercropping systems across N fertilization levels ranging from 0 to 300 kg N ha<sup>-1</sup> (Li et al., 2009). This is possibly because cereals can stimulate nodulation and N<sub>2</sub> fixation efficiency of legumes through competition for nitrate or ammonium which can inhibit nitrogen fixation (Corre-Hellou et al., 2006). In our study, lower soil  $\delta^{15}\text{N}$  in faba bean-based intercrops compared to sole faba bean suggests that intercropping increased biological N fixation, and consequently led to increased soil N input (Chapter 4). This mechanism may also occur in grasslands, where grasses coexist with legumes (Fornara and Tilman, 2008). We have demonstrated that, in systems without legumes, plant species diversity can enhance soil N storage. This was demonstrated in grasslands without legumes (Chapter 2) as well as in wheat/maize intercropping system (Chapter 4). There are several possible mechanisms: First, species-diversified systems may capture more N because of spatial and temporal differences in nutrient uptake (Hooper et al., 2005). Complementary N uptake in space can occur between shallow-rooted (e.g. *Anthoxanthum odoratum*) and deep-rooted (e.g. *Plantago lanceolata*) plants (Berendse, 1981). Complementary N uptake in time can occur between early-growing wheat and late-growing maize in wheat/maize intercrop. Second, this variation in species phenology may

also lead to a more efficient N recycling because mineralized N from decomposing roots of early species may be utilized by later species. Finally, these mechanisms may contribute to more root biomass in species-diversified system, which has been demonstrated in both grasslands and intercropping systems (Chapter 2 and 4). Increased root biomass, in turn, may further contribute to increased N uptake (Fornara & Tilman 2009), thereby potentially reducing N losses through leaching and/or denitrification. Collectively, these mechanisms imply that combining plant species with functionally complementary traits may not only enhance biomass production, but also improve soil fertility and help mitigate climate change (Fig. 1C).

Another question which this thesis has addressed is which mechanisms can explain the positive effect of plant species diversity on SOC decomposition in the two systems (Chapter 2 and 5; Dijkstra et al., 2005; Reid et al., 2012). Our results show that the decomposition of standard compost was similar across the different levels of plant species diversity in both systems (Chapter 3 and Chapter 5). This indicates that enhanced relative SOC decomposition is due to changes in SOM quality, rather than changes in decomposition microenvironment. The changes in SOM quality may be related to increased fresh organic matter input into soil, as a consequence of greater biomass production in diversified systems compared to monocultures. This may have led to a greater proportion of labile organic matter in the total SOM, reducing the total recalcitrance of SOM, thereby leading to an acceleration of the relative decomposition rate of SOC. This mechanism is consistent with the findings by Dijkstra et al. (2005) and Reid et al. (2012), who showed that plant species richness increased the relative decomposition rate of SOC and attributed this increase to enhanced biomass production. Our results regarding the decomposition of a standard compost indicate that plant diversity did not change the decomposition microenvironment in terms of either abiotic or biotic factors. However, plant diversity has been found to affect soil microbial biomass, activity and microbial community structure (Zak et al., 2003; Song et al., 2007; Eisenhauer et al., 2010; De Deyn et al., 2011; Eisenhauer et al., 2011). The diversity-induced changes in soil microorganism communities may influence SOC decomposition but, unfortunately, I did not measure any of such biological variables in this study.



**Figure 6.1** Plant species diversity effects on soil C and N dynamics (A), possible mechanisms (B) and proposed species combination to enhance SOM (C). (A) Red arrows denote C flows; green arrows denote N flows. The thickness (thin, modest and thick) of arrows and boxes represent the magnitude (slight, modest and large) of the effects of plant species diversity on different processes or variables. Symbols “+”, “-”, “0” and “?” represent positive, negative, neutral and uncertain effect, respectively. (C) The selection of species combination may include one, two or multiple complementary functional traits. For example, maize and faba bean combination may include all four complementary traits.

The effects of plant species diversity on decomposition of fresh root litter were not straightforward across the two systems. In the grassland experiment, root litter decomposition increased with increasing plant species richness under laboratory conditions (Chapter 3). In the intercropping experiment, wheat/maize intercropping decreased root litter decomposition by 33% compared to monocropping in the laboratory, whereas maize/faba bean increased it by 6% in the field (Chapter 5). The variable directions and magnitudes of the positive diversity effects on root litter decomposition found in both systems are essentially determined by the relative impacts of changes in litter mixing and change in decomposition microenvironment. In both systems, our results showed that the changes in soil decomposition microenvironment have a dominant effect on root litter decomposition, while litter mixing generally has a minor to no effect on root litter decomposition. This finding is consistent with results of previous work conducted in the Cedar Creek biodiversity experiment (Fornara et al., 2009).

Collectively, the results across the two systems suggest that diversity-induced changes in SOM quality predominantly affect the decomposition of SOC. In contrast, plant species diversity influences the decomposition of fresh root litter probably mainly through a soil-mediated effect.

### ***Estimating soil C sequestration potential***

In the face of rising atmospheric CO<sub>2</sub> concentration, it is crucial to identify effective strategies to mitigate climate change. Soil C sequestration has been recommended as a cost-effective and environmentally friendly strategy to offset greenhouse gas emissions over a relatively short period of 20 to 50 years (Lal, 2004). Soil C sequestration can be achieved through judicious land use and recommended management practices such as conservation tillage, cover crops, crop rotations, manuring, agroforestry, etc. (Albrecht and Kandji, 2003; Jarecki and Lal, 2003; Lal, 2004). These practices led to soil C sequestration rates ranging from 20-1000 kg C ha<sup>-1</sup> year<sup>-1</sup> (West and Post, 2002; Lal, 2004). To our knowledge, the soil C sequestration potential of intercropping has never been assessed. Such assessment is necessary to allow comparison of its C sequestration potential to other management practices to aid development of policy.

Long-term field experiments are an effective means to estimate the soil C sequestration potential of agricultural management practices. However, the number of existing long-term field experiments is limited. Setting up new experiments is costly and a considerable time period is required to detect changes in SOM. Models of the decomposition of organic matter have been developed to quantitatively describe soil C dynamics. Once a model has been validated, it can be used to extrapolate experimental results for the prediction of long-term SOC dynamics. Some models for SOC dynamics have been proposed. They can mainly be divided into two categories: mono-component models with a time-dependent relative decomposition rate, such as the model developed by Yang and Janssen (2000), and multi-component models in which each C pool (“component”) has a constant relative decomposition rate, such as the Century model (Parton, 1996).

The model of Yang and Janssen (2000) is defined by the equation:

$$Y_t = Y_0 \exp(-R_9 (ft)^{1-S})$$

where  $Y_t$  and  $Y_0$  are the amount of organic C or root mass present at time  $t$  and time 0, respectively.  $R_9$  is the relative decomposition rate in the first time step at a temperature of 9 °C.  $S$  is a measure of the speed at which the relative decomposition rate decreases over time. Greater values of  $S$  signal faster decline in decomposition rate.  $f$  is a temperature correction factor.

The Yang and Janssen model is here used to predict and compare the long-term SOC dynamics in monocropping versus intercropping systems, and to assess the soil C sequestration potential of intercropping. I chose this model because (1) the model contains only a few parameters, allowing for an easier calibration; (2) the model described the decomposition dynamics of root litter satisfactorily and parameters have been calibrated (Chapter 5); (3) the model has been successfully applied to analyse the impact of farming practices on SOM dynamics in northern China (Yang and Janssen, 1997).

Two scenarios (“Low” and “High”) are considered here to estimate the minimum and maximum soil sequestration potential of intercropping compared to monocropping, respectively. The “Low” and “High” scenarios are based upon the smallest and largest differences in aboveground biomass between maize/faba bean intercropping and

monocropping observed in the Wuwei field experiment from 2003 to 2006 (Li et al., 2007). The annual root C input was estimated by multiplying aboveground biomass under the two scenarios by several conversion coefficients including root: shoot ratio, root C content (%) and percentage of root biomass in upper 20 cm. The parameters ( $R$ ,  $S$  and  $f$ ) of the Yang and Janssen model were calibrated using the litter-bag experiment in the field reported in Chapter 5. All parameters and their sources are shown in Table 1. The initial soil C content was set to zero, then C accumulation of “new” organic matter in soil was estimated by adding the remaining quantities from each year (i.e.  $Y_1 + Y_2 + Y_3 + \dots + Y_n$ ). This equation cannot be integrated and it is therefore not possible to calculate a closed form solution for the steady-state SOM content. But after 50 years of constant supply of organic matter annually, SOM virtually reached equilibrium in both monocropping and intercropping (Fig. 2).

In the Low scenario, maize/faba bean intercropping accumulated 2770 kg more C per hectare than monocropping in the top 20 cm depth after 50 years, while 6210 kg C ha<sup>-1</sup> was additionally accumulated by intercropping in the High scenario. In comparison to monocropping, rates of soil C sequestration in intercropping ranged from 55 to 124 kg C ha<sup>-1</sup> year<sup>-1</sup> over the period of 50 years. This soil C sequestration potential is comparable to that of crop rotations with C sequestration rates in the top 10 to 30 cm depth ranging from 50-200 kg C ha<sup>-1</sup> year<sup>-1</sup> (West and Post, 2002; Lal, 2004). However, it is lower than that of no-till/conservation tillage which has a sequestration rate in the top soil of 100 to 500 kg C ha<sup>-1</sup> year<sup>-1</sup>. Assuming that one third of China’s cropland (~120,000,000 ha) may be used for intercropping (Zhang and Li, 2003), the C sequestration potential of intercropping compared to monocropping is estimated to be 2.2-5.0 Tg<sup>1</sup> C year<sup>-1</sup>, which accounts for only 0.1%-0.3% of total greenhouse gas emission (6100 Tg CO<sub>2</sub>-eq) in China for 2004 (Zhang et al., 2013b). However, these estimates are based on roots being the only sources of organic matter input into soil. If straw residues were also incorporated into soil, we would expect a higher soil C sequestration rate in intercropping because of its higher biomass production, meaning more residue production than in monocropping systems. This leads to the conclusion that intercropping can make a small, but still non-negligible, contribution to mitigate greenhouse gas emission.

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<sup>1</sup> 1 Tg = 10<sup>12</sup> g



**Table 6.1** Parameters used for estimating long-term soil C accumulation in intercropping and monocropping systems.

Parameters	Unit	Monocrop maize	Monocrop faba bean	Maize/faba bean intercrop
Aboveground biomass <sup>1</sup> (Low scenario) (High scenario)	kg/ha	28546 23077	7754 7954	21769 (Maize:17476; Faba bean:4293) 25298 (Maize:19919; Faba bean:5379)
Root: shoot ratio <sup>2</sup>	/	0.55	0.62	Maize: 0.55; Faba bean: 0.62
Root C content <sup>3</sup>	/	0.41	0.42	Maize: 0.42; Faba bean: 0.41
% Root biomass (in upper 20 cm) <sup>3</sup>	/	0.61	0.55	Maize: 0.63; Faba bean: 0.57
$R^4$	year <sup>S-1</sup>	0.67	0.52	0.63
$S^4$	/	0.41	0.46	0.43
$f^5$	/	0.99	0.99	0.99

<sup>1</sup>Data are from the long-term experiment (Li et al., 2007). The Low and High scenarios are based upon the smallest (20%) and largest (63%) differences in aboveground biomass between maize/faba bean intercropping and monocropping observed in 2003 and 2006, respectively.

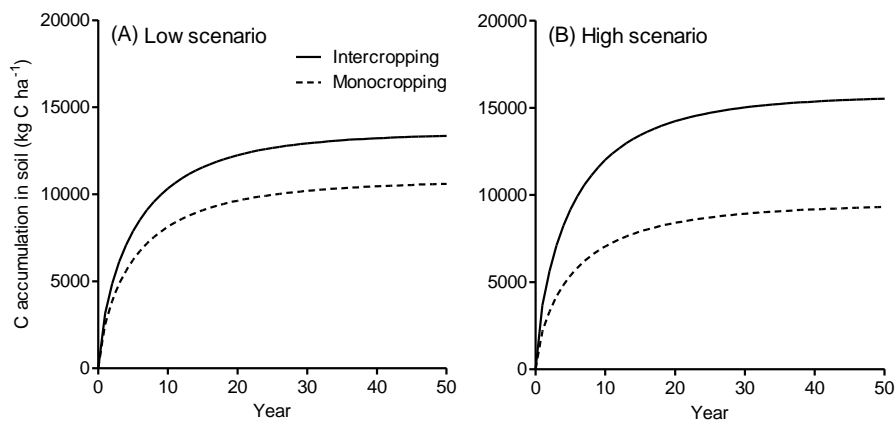
<sup>2</sup>Data in monocrop maize and faba bean are from Johnson et al. (2006). Since our results showed that intercropping did not significantly affect the ratio of root biomass to aboveground biomass of maize or faba bean (Chapter 4, this thesis), we use the same values for intercropped maize and faba bean.

<sup>3</sup>Data are from a short-term field experiment close to the long-term field experiment (Chapter 4, this thesis).

<sup>4</sup>The Yang and Janssen model is calibrated according to a litter-bag experiment conducted in the long-term field experiment (Chapter 5, this thesis).

<sup>5</sup>The parameter  $f$  is calculated based upon the equation  $f = 0.1 * (T + 1)$ , when  $T$  ranges from -1 °C to 9 °C (Janssen, 1992). The annual average temperature in this long term experiment is 8.9 °C.

Grasslands/grazing lands also have a high soil C sequestration potential (Lal, 2004). Grassland management practices intended to increase forage production may potentially increase SOM (Jones and Donnelly, 2004). Adoption of management practices including fertilization, irrigation, sowing of legumes and grasses, or introducing earthworms has led to the increases in rates of C sequestration ranging from 110-3040 kg C ha<sup>-1</sup> year<sup>-1</sup>, with a mean of 540 kg C ha<sup>-1</sup> year<sup>-1</sup> (Conant et al., 2001). Furthermore, increasing plant species diversity in grasslands can substantially contribute to increasing C storage in soil. For example, in the Cedar Creek biodiversity experiment, plots with 16 plant species sequestered 556 kg C ha<sup>-1</sup> year<sup>-1</sup> more than plots with only one species, over a 12-year period (Fornara and Tilman, 2008). In the Jena biodiversity experiment, 1170 kg C ha<sup>-1</sup> year<sup>-1</sup> was additionally sequestered in 16-species mixture plots compared to monoculture



**Figure 6.2** C accumulation in the top 20 cm soil depth for maize/faba bean intercropping and monocropping in the long-term field experiment over 50 years in the Low (A) and High (B) scenarios. C accumulation in monocropping is averaged across monocrops of maize and faba bean. The Low and High scenarios represent the minimum and maximum soil C sequestration potential of intercropping compared to monocropping. The scenarios are based upon the smallest and largest differences in aboveground biomass between intercropping and monocropping observed in the Wuwei field experiment from 2003 to 2006. C accumulation is estimated using the mono-component model developed by Yang and Janssen (2000). The values of all parameters are shown in table 6.1.

plots over a 4-year period. Much of this increase was attributed to the presence of legumes and/or C4 grasses. Indeed, sowing the legume species *Trifolium pratense* in a 16-year grassland experiment in the UK led to the additional sequestration of approximately 670 kg C ha<sup>-1</sup> year<sup>-1</sup> (De Deyn et al., 2011). In the Wageningen biodiversity experiment, which does not include any legumes, we found that the 8-species mixture plots stored 185 kg C ha<sup>-1</sup> year<sup>-1</sup> more than monocultures over an 11-year period (Chapter 2). This suggests that restoration of grassland biodiversity will increase soil C sequestration even in the absence of legumes. Interestingly, high diversity plots in grassland biodiversity experiments show greater increases in the rate of C sequestration than intercropping despite their productivity being lower. One possible explanation for this is that the roots of perennials contain comparatively more recalcitrant substances and decompose in a less-disturbed soil environment than those of annuals do (Cadisch and Giller, 1997; Wardle et al., 1997), thereby leading to a higher C conversion efficiency. Furthermore, the

comparatively high soil N levels in the intercropping experiment as compared to those in grassland biodiversity experiments may lead to a faster decomposition of organic matter.

### ***Implication for long-term soil fertility***

Apart from mitigating climate change, another important benefit of soil C sequestration lies in improving soil fertility and thus advancing food security (Lal, 2004). Enhancing SOM content can generally improve soil physical properties (e.g. increasing water-holding capacity, and mitigating soil compaction to facilitate root growth), promote the activity of soil biota, and enhance nutrient availability thereby supporting plant growth (Troch and Thompson, 2005). Crop diversification within agroecosystems is being increasingly promoted as a plausible strategy for sustainable agriculture (Matson et al., 1997; Tilman et al., 2002; Bommarco et al., 2013). It is well established that crop rotation as a temporal diversification strategy enhances soil fertility (Jarecki and Lal, 2003; McDaniel et al., 2014). Can intercropping as a spatial diversification increase soil fertility? A number of studies have shown that cereal/legume intercrops can improve soil fertility because legumes can bring additional atmospheric N into systems through biological N fixation (Lithourgidis et al., 2011). This is especially important in agroecosystems with reduced N fertilizer to minimize environmental impact, e.g. in Europe (Jensen et al., 2010), or agroecosystems where availability of nitrogen fertilizer is limited, e.g. in Africa (Snapp et al., 2010). Moreover, our results show that cereal/cereal intercrops can also improve soil fertility to a similar extent as cereal/legume intercrops (Chapter 4). This is probably due to greater retention of N sources from fertilizer as well as more efficient N recycling. Efficient N retention and recycling are crucial in high-input and high-output agroecosystems (e.g. in China) where substantial food production is required to meet increasing population and increasing food demand per capital (Zhang et al., 2013a). Cereal/legume intercrops are also important in these high-input and high-output agroecosystems in the context of partly substituting N from fertilizer with biologically fixed N by legumes. This will reduce fertilizer input and thus help mitigate the associated environmental impacts while maintaining high food production.

Biodiversity experiments in grasslands have shown that the positive effects of biodiversity on productivity increase over time (Cardinale et al., 2007). One of the potential

mechanisms is through positive ecosystem feedbacks, associated with C and N cycling (Reich et al., 2012). Indeed, our results show that plant species diversity enhanced soil C and N storage and also increased net potential N mineralization rate (Chapter 2). The latter is likely to fuel a further increase of primary production through increased N availability. This probably explains the strengthening biodiversity-productivity relationship we observed in the last three years (Chapter 2). Does this mechanism occur in intercropping agroecosystems? Unfortunately, this question cannot be answered because the potential N mineralization rate in intercropping was not measured, and data of agricultural biomass production in the last years of the Wuwei long-term field experiment are not yet available. Nevertheless, enhanced soil C and N storage and SOC decomposition probably indicate a positive ecosystem feedback on long-term intercropping productivity. Hence, intercropping may be a promising strategy to improve agroecosystems services by simultaneously enhancing crop production, improving soil quality and mitigating greenhouse gas emission.

### ***Future research challenges***

In conjunction with previous findings, our work shows that plant species diversity enhances soil C and N storage under plant communities both including and excluding legumes. This provides further evidence of the importance of conserving plant species diversity (Isbell et al., 2011; Cardinale et al., 2012). From the practical perspective, it is impossible to conserve all plant species because the cost for conserving diverse species is high (Naidoo and Ricketts, 2006). Future research may additionally consider which species or combinations of species are most effective at promoting multiple or specific ecosystem services. As reported in this thesis, a forb species *Centaurea jacea* enhanced plant productivity and soil C storage (Chapter 2). This is consistent with results from the “Jena” experiment, which showed that the presence of tall forb species (including *Centaurea jacea*) reduced soil C losses. Moreover, Fornara and Tilman et al. (2008) showed that the joint presence of legumes and C4 grasses can substantially increase root biomass, and soil C and N accumulation. This information helps to shed some light on designing the optimal intercropping systems to maximize the ecosystem services important for sustainable agriculture.

Although we demonstrate that soil N storage increases with plant species richness, we did not systematically examine soil N dynamics (especially N losses) in both systems in this thesis. Several grassland biodiversity experiments have explored the effects of plant diversity on inorganic N leaching and reported mixed results: Oelmann et al. (2007) and De Deyn et al. (2009) showed that increasing plant species richness decreases N leaching probably through increased plant N uptake. Conversely, Scherer-Lorenzen et al. (2003) and Phoenix et al. (2008) found no effect of plant species richness. Apart from inorganic N leaching, leaching losses of dissolved organic N can be relatively large (Murphy et al., 2000). Dijkstra et al. (2007) showed that plant species diversity increased leaching loss of organic N, possibly through increasing the size of organic N pool and/or stimulating microbial activity. Weigelt et al. (2005) found that plant species differed in their uptake capacity of inorganic and organic N, suggesting that species diverse mixtures may reduce leaching loss of total N (inorganic and organic N) through complementary use of N of different forms. Further studies should comprehensively explore the effects of plant species diversity on leaching losses of the total N as well as other N loss pathways (e.g. denitrification) in grasslands. Studies in agroecosystems, where N fertilizer is rarely applied, have shown that intercropping decreases nitrate leaching and nitrous oxide emissions (Hauggaard-Nielsen et al., 2003; Pappa et al., 2011). Nevertheless, the magnitude of the effect is small, probably because of low N input. In agroecosystems with high input of inorganic N fertilizer and irrigation water (e.g. in China), intercropping has been found to decrease soil mineral N accumulation in the soil profile through complementary N use (Li et al., 2005), possibly leading to less N leaching losses. I expect that intercropping can play a more important role in reducing N leaching losses in agroecosystems with higher rates of fertilization and irrigation. However, little is known regarding the effects of intercropping on N leaching in such systems. Further research is also required to explore how intercropping affects nitrous oxide emissions. This will allow a comprehensive evaluation of intercropping's potential to mitigate greenhouse gas emissions.

Our results show that plant species diversity enhances decomposition of SOM in both the Wageningen grassland experiment and the Wuwei intercropping experiment. These results are from a series of short-term experiments (not longer than 1 year). Nevertheless,

short-term diversity effect on decomposition dynamics do not necessarily lead to long-term effects on soil C storage if plant diversity may affect stabilization mechanisms of soil organic matter. SOC can be stabilized in soil through physical protection, chemical stabilization and biochemical stabilization (Six et al., 2002). For example, physical protection can occur through aggregation, whereby SOC can become encased within a soil aggregate which then forms a physical barrier between SOC and extracellular enzymes released by soil microorganisms, thereby reducing microbial decomposition. Rhizodeposits and microbial products have been found to act as cementing agents, which were beneficial for aggregate formation (Oades, 1984). Increased soil C input in diversified plant/crop communities may increase soil microbial biomass and microbial activity (Zak et al., 2003; Chung et al., 2007; Song et al., 2007; Eisenhauer et al., 2010), and consequently more aggregate formation and physical protection of SOC. Nevertheless, there may also be a trade-off whereby increased soil microbial biomass and activity will lead to enhanced SOC decomposition. Future research could address how plant species diversity affects these stabilization mechanisms in the long term.

A large body of literature has reported the effects of intercropping on only a few ecosystem services including crop yields enhancement, pest and disease control, and mitigation of complete crop failure (Lithourgidis et al., 2011). It is necessary to evaluate more ecosystem services to allow a comprehensive evaluation of the benefits or costs of intercropping. For example, intercropping generally produces more straw residues. This may potentially contribute to greater energy production if straw residues are used for biofuel production. Moreover, previous studies focused on examining the individual ecosystem services over the short term, without consideration of the trade-offs or synergies that can arise when considering a broad suite of ecosystem services in a long term (Werling et al., 2014). Our study implies that intercropping can simultaneously enhance short-term crop yields and long-term soil fertility. The accumulation of soil C and N may further benefit crop productivity in the long term via positive ecosystem feedbacks.

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## Summary

The soil carbon (C) stock comprises more than two-thirds of terrestrial C globally. Thus, changes in the pool size of soil C have important consequences for the global C cycle. By converting natural vegetation into agricultural land, humans have depleted soil organic C by 10%-59%. Such decrease has led to a rise in atmospheric CO<sub>2</sub> concentration, which has probably contributed to global warming and related environmental problems. Another hazard is that the decrease in soil organic C results in a reduction of soil fertility. This is posing a tremendous challenge for mankind to meet a doubling of global food production projected for the next 50 years. Recently, sequestering C into soil has been promoted as a cost-effective and environmentally friendly strategy to help mitigate climate change over a short period of 20 to 50 years, and more importantly, to achieve food security through improvement in soil quality in agroecosystems.

The concern that loss of biodiversity may be detrimental to the functioning of ecosystems has aroused considerable interest in exploring the relationship between biodiversity and ecosystem functioning. Most biodiversity studies have focused on the effects of biodiversity on biomass production, and showed that primary production generally increases with increasing biological diversity. These findings imply that more diverse ecosystems may sequester more C into soil as a consequence of increased biomass input. Temperate grasslands, occupying about one third of the Earth's natural vegetation, is one of the natural ecosystems with a high soil C sequestration potential. A few grassland biodiversity experiments have demonstrated that plant species diversity promotes C and N accumulation through increased soil C and N input resulting from enhanced root biomass production. These studies also showed that the presence of legumes enhances productivity, soil C and N sequestration. Legumes can additionally access atmospheric N via symbiotic N fixation. As most natural grasslands are N-limited, the increased probability of legumes being included in more diverse communities will enhance N input into soil, thereby increasing productivity, and soil C and N sequestration. Hence, it is unclear whether plant species diversity can promote soil C and N storage in the absence of legumes, and which mechanisms, if any, may drive this effect.

## Summary

In this research, I used an 11-year grassland biodiversity experiment without any legume species to determine the effects of plant species diversity on soil C and N storage, and above- and below-ground biomass production. I found that also in the absence of legumes, plant species diversity promotes soil C and N storage after 11 years (Chapter 2). Soil C and N storage increased by 18% and 16% in eight-species mixtures compared to the average of monocultures of the same species, respectively. Increased soil C and N storage were explained by increased C input and N retention, resulting from enhanced productivity. In addition, the presence of a forb species (i.e. *Centaurea jacea*) in species-diverse plots enhanced soil C storage, which may also have contributed to the positive diversity effect on soil C storage.

Some studies suggest that plant species diversity enhances decomposition of organic matter. This may partly or fully compensate the advantages of plant species diversity for soil C sequestration through increased C input into soil resulting from enhanced productivity. I collected soil samples from the 11-year experiment and incubated them in the laboratory over 145 days to determine the diversity effect on soil organic C (SOC) decomposition. I found that plant species diversity increased the relative decomposition rate of SOC (Chapter 2). Such increase was mainly explained by increased biomass production. Plant species diversity can potentially affect organic matter decomposition through either changes in organic matter quality or changes in decomposition microenvironment (including abiotic and biotic factors). Thus, it is probable that increased productivity (thus more fresh organic matter inputs) have led to greater proportion of labile C in soil, reducing the recalcitrance of soil organic matter (SOM), thereby accelerating the rate of decomposition. Moreover, I observed a similar decomposition rate of standard compost added to soils that were planted with different number of plant species (Chapter 3). This further confirms that enhanced relative SOC decomposition rate is related to changes in SOM quality, rather than changes in soil decomposition microenvironment. I further examined the effect of plant diversity on decomposition of fresh root litter in a laboratory incubation experiment (Chapter 3). I found that plant diversity enhanced decomposition of root litter via changes in decomposition microenvironment, but not via changes in litter mixing. Obviously,

regardless of SOC or fresh root litter, accelerated decomposition that led to greater C loss did not surpass increased C input from enhanced productivity.

Several studies have shown that the positive diversity effect on biomass production increases over time. Possible explanations include increased functional turnover in species and reduced negative impact of soil biota at high diversity. I hypothesized that enhanced soil C and N storage leads to a positive feedback to productivity, associated with accelerated C and N cycling. I measured potential net soil N mineralization rate and found that soil N mineralization rates increased with plant species diversity. In conjunction with enhanced soil C and N storage, this can explain the strengthening of the positive diversity-productivity relationship observed in the last years of this experiment (Chapter 2).

Concerns about sustainability in agricultural systems stimulate much interest in exploring ecologically-based management practices to achieve food security, while reducing the environmental impact. Among these practices, crop diversification is being increasingly promoted as a plausible strategy for ecological intensification. For example, intercropping, i.e. the simultaneous cultivation of two or more crop species in the same field, is an approach to increase diversity in agroecosystems. Intercropping generally increases aboveground productivity. Such increase is attributed to temporal and spatial complementarity in resource acquisition between species, or facilitation, and/or reduction in the impacts of pests, diseases and weeds. Recent studies suggest that intercropping may also enhance belowground productivity, but it has never been demonstrated. Understanding the influence of crop diversification on soil C sequestration and soil fertility is critical for strengthening the ecological foundations of sustainable agriculture. The benefits of crop rotation and cover cropping on enhancements of soil C and N storage are well-established. However, there were no reports on long-term effects on the storage of soil C and N under intercropping. Some work has been done, but the duration of those studies was too short to demonstrate sequestration of organic C and N.

In this research, I used a 7-year intercropping field experiment, the oldest one of its kind in existence today, to determine the effects of intercropping on soil C and N storage. Our

## Summary

results, for the first time, demonstrated that intercropping enhances soil C and N storage by 4% and 11%, respectively (Chapter 4). Two 1-year field experiments consistently showed that intercropping increased grain yield and aboveground biomass. More importantly, intercropping also enhanced peak root biomass on average by 23% compared to monocropping, providing a plausible explanation for the observed soil C sequestration (Chapter 4). Our results showed that faba bean-based intercropping (i.e. maize/faba bean and wheat/faba bean) decreased soil  $\delta^{15}\text{N}$  values compared to single cropping with faba bean. This suggests that intercropping may increase soil N storage through increased soil N input resulting from enhanced biological N fixation (Chapter 4). However, the increase in soil N storage also occurred in the maize/wheat intercropping system, highlighting the contributions from a broader suite of mechanisms to N retention. For example, differences in rooting depths and differences in crop phenology between intercropped wheat and maize probably have contributed to more N acquisition. In addition, this variation in species phenology could also lead to a more efficient N recycling because mineralized N from decomposing roots of early growing wheat may be utilized by later maize.

Our results showed that enhanced standing root biomass (23%) by intercropping led to a small increase in soil C storage (4%) after 7 years. To examine the intercropping effect on SOC decomposition, I incubated soil samples collected from the 7-year experiment in the laboratory over 112 days. I found that intercropping indeed increased the relative decomposition rate of SOC (Chapter 5). This accelerating effect of intercropping was attributed to the changes in SOM quality, not to the changes in decomposition microenvironment. I further performed experiments in the laboratory and in the field to unravel if and how intercropping affects decomposition of fresh root litter: through changes in soil decomposition microenvironment and/or through changes in litter diversity (Chapter 5). I found that these intercropping effects depended on incubation conditions and crop combination. In the laboratory, maize/wheat intercropping decreased root litter decomposition through a soil-mediated effect. In contrast, maize/faba bean intercropping accelerated root litter decomposition in field conditions via a weak but significant litter diversity effect. The reduction in decomposition of root litter in soil from maize/wheat intercropping system may be attributed to lower nutrient

availability due to greater crop N uptake in intercrops than that in monocrops. The positive litter diversity effect found in maize/faba bean intercrop is probably related to N transfer from high-N root litter of faba bean to low-N root of maize, whereby accelerating the decomposition rate of maize root.

In conclusion, a general picture emerges that the positive effects of plant species diversity on several ecosystem functions (i.e. soil C and N storage, biomass production and SOC decomposition) reported for perennial N-limited grasslands are paralleled in annual N-rich intercropping agroecosystems. The increase in soil C storage in both systems is attributed to increased soil C input through biomass production, partly compensated by enhanced SOC decomposition. The increase in soil N storage is ascribed to increased soil N input in systems with legumes or increased N retention in systems without legumes. Our modelling work show that intercropping has a soil C sequestration potential comparable to other commonly recommended practices (e.g. rotation) and can make a small, but still non-negligible contribution to mitigating greenhouse gas emissions. More importantly, intercropping may improve soil fertility and benefit long-term ecosystem productivity. Hence, intercropping may be a promising strategy to improve agroecosystems services by simultaneously enhancing crop production, improving soil quality and mitigating greenhouse gas emissions. Future researches may address the mechanisms underlying the effects of plant species diversity on long-term SOC dynamics as well as systematically investigate plant-soil N dynamics (e.g. denitrification) in both species-diverse grasslands and intercropping agroecosystems. This will allow a comprehensive evaluation of the potential of plant species diversity in greenhouse gas mitigation.





## Samenvatting

De koolstof (C) voorraad in de bodem vormt meer dan twee derde van de totale C voorraad op aarde. Veranderingen in de omvang van deze bodemvoorraad hebben daarom belangrijke consequenties voor de C cyclus op aarde. Door menselijk handelen (verandering in van natuurlijke vegetatie naar landbouw) is de organische C voorraad in de bodem met 10-59% afgenomen, afhankelijk van de landgebruiksverandering. Dit heeft geleid tot een toename van de CO<sub>2</sub> concentratie in de atmosfeer. Deze toename heeft bijgedragen aan opwarming van de aarde en de daaraan gerelateerde milieuproblemen. Een ander risico van de vermindering van de organische C voorraad in de bodem is vermindering van de bodemvruchtbaarheid. Mede hierdoor wordt de opgave om de voedselproductie de komende 50 jaar te verdubbelen, een grote uitdaging. Vastlegging van C in de bodem kan een goedkope en milieuvriendelijke maatregel zijn die bijdraagt aan vermindering van effecten van klimaatverandering op een termijn van 20 – 50 jaar. Deze maatregel kan bovendien bijdragen aan voedselzekerheid doordat ze de bodemkwaliteit in landbouwsystemen verhoogt.

De veronderstelde achteruitgang in biodiversiteit heeft geleid tot een toenemende aandacht voor de relatie tussen het functioneren van ecosystemen en hun biodiversiteit. Veel biodiversiteitsstudies hebben zich gericht op de effecten van biodiversiteit op primaire productie. Ze hebben aangetoond dat de primaire productie meestal toeneemt met toenemende diversiteit. Deze resultaten impliceren dat meer diverse ecosystemen meer C in de bodem zouden kunnen vastleggen als gevolg van een grotere biomassa productie. Graslanden van gematigde streken kunnen potentieel veel C vastleggen in de bodem: ze vormen namelijk een derde van de natuurlijke vegetatie op aarde. In enkele grasland biodiversiteitsexperimenten is aangetoond dat plantensoortenrijkdom ophoping van C en N (stikstof) stimuleert via hogere wortelproductie. Deze experimenten hebben ook aangetoond dat vlinderbloemigen een belangrijke rol spelen bij het verhogen van de productiviteit en deze vastlegging van C en N in de bodem. Vlinderbloemigen kunnen atmosferische N binden via een symbiose met N-bindende bacteriën. In de meeste natuurlijke graslanden wordt de primaire productie beperkt door de beschikbaarheid van N. Omdat de kans op aanwezigheid van leguminosen groter is in meer diverse plantengemeenschappen, leidt een grotere soortenrijkdom tot hogere primaire productie en meer

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vastlegging van C en N in de bodem. Het was niet bekend of grotere soortenrijkdom zonder vlinderbloemigen ook leidt tot meer vastlegging van C en N, en zo ja, welke mechanismen hiervoor verantwoordelijk zijn.

In dit proefschrift heb ik in een 11-jarig grasland biodiversiteitsexperiment onderzocht wat het effect is van soortenrijkdom op vastlegging van C en N in de bodem en op boven- en ondergrondse productie. Ik heb vastgesteld dat soortenrijkdom ook in afwezigheid van vlinderbloemigen opslag van C en N in de bodem bevordert (Hoofdstuk 2). Bodem C en N waren met 18% respectievelijk 16% toegenomen in een mengsel van acht soorten ten opzichte van het gemiddelde van acht monocultures van dezelfde soorten. Deze toename kon worden verklaard door toegenomen toevoer van C naar de bodem als gevolg van toegenomen wortelproductie, en een verhoogde retentie van N door geringere verliezen. Verder verhoogde de aanwezigheid van echt knoopkruid (*Centaurea jacea*) de C opslag in de bodem, hetgeen heeft bijgedragen aan de positieve correlatie tussen diversiteit en C opslag.

Een aantal studies suggereert dat een grotere rijkdom aan plantensoorten de afbraak van organische stof versnelt. Dit kan geheel of gedeeltelijk het voordeel van groter C toevoer in de bodem door hogere productie teniet doen. Ik heb bodemmonsters in het 11-jarige grasland verzameld en gedurende 145 dagen in het laboratorium geïncubeerd om vast te stellen wat het effect is van diversiteit op afbraak van bodem organische C. Plantensoortenrijkdom bleek de relatieve decompositiesnelheid van bodem organische C te vergroten (Hoofdstuk 2). Deze toename kon verklaard worden door toename in biomassaproductie. Soortenrijkdom kan de afbraak van bodem organische stof op twee manieren beïnvloeden: via de verandering van de kwaliteit van de bodem organische stof of via veranderingen in de biotische en abiotische omgevingsfactoren die de afbraaksnelheid beïnvloeden. Toename van de biomassaproductie kan hebben geleid tot een grotere fractie van relatief vers en gemakkelijk afbreekbare organische C in de bodem, waardoor de bodem organische stof als totaal minder stabiel en sneller afbreekbaar is geworden. Wanneer compost werd gemengd met grond uit veldjes die verschilden in soortenrijkdom, verschilde de afbraaksnelheid van deze compost niet (Hoofdstuk 3). Dit bevestigde dat de hogere afbraaksnelheid in van bodem organische stof van soortenrijkere veldjes verklaard moet worden door verandering in de kwaliteit van deze organische stof, en niet door

veranderingen in de omgevingsfactoren. Verder heb ik het effect van diversiteit op de afbraaksnelheid van vers wortelmateriaal onderzocht (Hoofdstuk 3). Ook hier versnelde grotere soortenrijkdom de afbraaksnelheid, maar niet via veranderingen van de substraatkwaliteit maar juist via veranderingen in de omgevingsfactoren. Echter: de verhoogde afbraaksnelheid van zowel bodem organische stof als vers wortelmateriaal in soortenrijkere veldjes compenseerde de verhoogde C input door hogere biomassa slechts gedeeltelijk. Uiteindelijk trad er in diversere veldjes meer C vastlegging op.

Verschillende studies hebben laten zien dat het positieve effect van diversiteit op biomassa productie toeneemt in de tijd. Een mogelijke verklaring hiervoor is een versnelde successie en als gevolg daarvan een geringere invloed van pathogenen bij hogere diversiteit. Ik toetste de veronderstelling dat grotere opslag van C en N in de bodem positief terugkoppelt naar productiviteit via een versnelde omzetting van C en N. Ik heb netto N mineralisatie snelheid in de bodem gemeten en stelde vast dat deze toenam met plantensoortenrijkdom. Samen met de verhoogde vastlegging van C en N in de bodem kan dit verklaren waarom het positieve effect van diversiteit op biomassa toenam in het 11-jarig grasland experiment na verloop van tijd.

Zorgen over de duurzaamheid van landbouw systemen hebben geleid tot exploratie van op ecologische concepten gebaseerde management opties om voedselzekerheid te garanderen en tegelijkertijd het milieu te sparen. Gewasdiversificatie is één van deze opties die gepromoot worden als onderdeel van ecologische intensivering van de landbouw. Mengteelt (het tegelijkertijd verbouwen van twee of meer verschillende gewassen in hetzelfde veld) is een vorm van gewasdiversificatie. In het algemeen verhoogt mengteelt de opbrengst. Deze verhoging wordt toegeschreven aan complementariteit van de verschillende gewassen in tijd en ruimte in de benutting van hulpbronnen (licht, water, nutriënten), facilitatie, en/of vermindering van schade door ziekten, plagen en onkruiden. Recente studies suggereren dat mengteelt ook de ondergrondse productiviteit zou verhogen. Echter, dit was nog niet aangetoond. Voor de verduurzaming van de landbouw is het belangrijk te begrijpen hoe gewasdiversificatie de C vastlegging in de bodem beïnvloedt. De effecten van andere vormen van gewasdiversificatie (rotatie en vanggewassen) op C en N vastlegging in de bodem zijn bekend, maar van mengteelt

zijn deze nog niet bekend. De publicaties over deze effecten betreffen tot nu toe experimenten die te kort duurden om eventueel toegenomen C en N vastlegging te kunnen meten.

In dit proefschrift onderzocht ik effecten van mengteelt op opslag van C en N in de bodem in het, voor zover mij bekend, langstdurende veldexperiment over mengteelt ter wereld. Als eersten toonden wij aan dat mengteelt inderdaad opslag van C en N in de bodem verhoogt ten opzichte van monoculturen, namelijk met 4, respectievelijk 11% (Hoofdstuk 4). Twee eenjarige veldexperimenten lieten zien dat mengteelt de niet alleen de bovengrondse biomassa en graanopbrengst verhoogde, maar ook de wortelbiomassa. Deze was op het moment van bemonsteren 23% hoger in mengteelt van twee gewassen dan in het gemiddelde van de twee monoculturen van de samenstellende soorten. Dit was een logische verklaring van de verhoging van C opslag in de bodem onder mengteelt (Hoofdstuk 4). De percentages  $\delta^{15}\text{N}$  in bodemmonsters van mengteelten van veldboon met maïs of tarwe waren lager dan die van een monocultuur van veldboon, hetgeen erop duidt dat mengteelt de N opslag in de bodem verhoogt via biologische N fixatie. (Hoofdstuk 4). Echter, verhoging van N opslag in de bodem trad ook op in mengteelt van maïs en tarwe, dus waarschijnlijk dragen ook andere mechanismen bij. De verschillen in bewortelingsdiepte en fenologie tussen maïs en tarwe kunnen bijvoorbeeld hebben bijgedragen aan een betere retentie van N in het gewas-bodem systeem. Variatie in fenologie kan ook geleid hebben tot een N kringloop met minder verliezen omdat N dat mineraliseert tijdens de afbraak van de wortels van het eerst geoogste gewas (tarwe) kan worden opgenomen door het laatst geoogste gewas (maïs).

Wij hebben aangetoond dat de toename van de wortelbiomassa in mengteelt (23%) slechts leidde tot een geringe toename van C opslag (4%) na 7 jaar. Daarom heb ik ook het effect van mengteelt op bodem organische C afbraak onderzocht. Ik heb bodemmonsters uit het 7-jarig experiment gedurende 112 dagen geïncubeerd in het laboratorium en vond dat mengteelt de relatieve afbraaksnelheid van bodemorganische C inderdaad verhoogde (Hoofdstuk 5). Deze versnelde afbraak was het gevolg van veranderingen in substraat kwaliteit, en niet van veranderingen van de bodemfactoren. Verder heb ik in het veld en in het laboratorium onderzocht of de afbraak van vers wortelmateriaal door mengteelt werd beïnvloed, en zo ja, via de kwaliteit van het wortelmateriaal of via veranderingen in de omgeving waarin de afbraak

plaatsvindt. De effecten van mengteelt bleken af te hangen van de experimentele condities: in het laboratorium verlaagde mengteelt van maïs en tarwe de afbraaksnelheid van wortelmateriaal door een verandering in de omgeving (de bodem). Echter, in het veld verhoogde mengteelt van maïs en veldboon de afbraak van wortelmateriaal door een klein maar significant effect op kwaliteit van het substraat. De verlaging van de afbraaksnelheid in de maïs/tarwe combinatie via de bodem is mogelijk een gevolg van lagere N beschikbaarheid doordat het gemengde gewas meer N opneemt dan een monocultuur. De verhoogde afbraaksnelheid van wortelmateriaal in de maïs/veldboon mengteelt kan komen door N overdracht (*transfer*) van N-rijker wortelmateriaal van veldboon naar dat van maïs, waardoor de afbraak van maïs wortelmateriaal werd versneld.

Dit alles leidt tot de conclusie dat de positieve effecten van plantensoortenrijkdom op ecosysteemdiensten van de bodem (opslag van C en N, biomassa productie en afbraak van organische stof) zoals die zijn waargenomen in N-gelimiteerd graslanden met overblijvende soorten, ook voorkomen in N-rijke landbouwsystemen met eenjarige soorten. De toename van C opslag in de bodem in beide systemen is een gevolg van hogere biomassa productie. De hogere toevoer van organisch C naar de bodem wordt gedeeltelijk teniet gedaan door een hogere afbraak ervan. De toename van N opslag in de bodem wordt veroorzaakt door een hogere vastlegging van atmosferische N in systemen met vlinderbloemigen en/of geringere N verliezen. Mengteelten hebben een potentie tot C vastlegging die in omvang vergelijkbaar is met andere landbouwpraktijken die C vastleggen (bijv. rotatie). Mengteelt kan daardoor een kleine maar niet verwaarloosbare bijdrage leveren aan vermindering van effecten van broeikasgas uitstoot. Bovendien, en wellicht belangrijker, kan mengteelt de bodemvruchtbaarheid verbeteren en op lange termijn de productiviteit verhogen. Kortom: mengteelt kan een veelbelovende strategie zijn die agroecosysteemdiensten verbetert doordat tegelijkertijd bodemkwaliteit wordt verbeterd en wordt bijgedragen aan het beheersen van klimaatverandering. Er is meer onderzoek nodig naar de mechanismen die ten grondslag liggen aan het effect van plantensoortenrijkdom op bodem organische C dynamiek, met name de rol van biologische processen en bodembiota. Bovendien behoeven de verschillende mechanismen voor verbeterde N retentie in soortenrijke graslanden en in mengteelten nader onderzoek. Nader onderzoek kan leiden tot het identificeren van opties voor het benutten van plantensoortenrijkdom voor vastlegging van C en verbetering van bodemkwaliteit.



## 摘要

土壤碳库占据超过三分之二的全球陆地碳库。因此，土壤碳库的变化对于全球碳循环有着重要的影响。由于大量自然植被转变成农业用地，人类已经耗尽了10%-59%的土壤碳库。土壤碳库的耗竭导致了大气二氧化碳浓度的升高，这很可能加剧了全球变暖以及相关的环境问题的恶化。土壤碳耗竭的另一个危害是造成土壤肥力的下降。这对于人类要满足未来50年加倍的粮食生产提出了巨大挑战。近年来，土壤碳固定作为一种经济有效、环境友好的策略被推广用以帮助减缓未来20-50年的环境恶化，更重要的是，它被用来提升农田生态系统的土壤质量，实现粮食安全。

对于生物多样性损失可能有害于生态系统功能的担忧引起了人们极大的兴趣去探索生物多样性与生态系统功能的关系。大部分生物多样性的研究关注物种多样性对于生物量生产的影响，并且发现初级生产力一般随着生物多样性的增加而增加。这些发现暗示着多物种的生态系统由于生物量投入的增加可以在土壤中固定更多的碳。约占地球自然植被三分之一的温带草原是土壤碳固定潜力高的自然生态系统之一。几个草地生物多样性试验发现植物多样性增加土壤碳、氮的累积。这是由于多样性增加了根系生物量，进而增加了土壤有机碳、氮的投入。这些研究也发现了豆科植物的存在能提高植物生产力以及土壤碳、氮的固定。豆科植物可以通过生物固氮的途径来获取大气中的氮。由于氮是大部分自然草原植被生长的限制因子，而多物种的植物群落包含豆科作物几率更大，因此可以向土壤中投入更多的氮，并最终提高植物生产力及土壤碳、氮的固定。因此，在不存在豆科植物的情况下，植物多样性是否可以增加土壤碳、氮储量？如果增加的话，是什么机制驱动着这种变化？这些问题目前还尚不清楚。

本研究使用了一个11年不包含豆科植物的草地生物多样性试验，来测定植物多样性对于土壤碳、氮储量以及地上、地下生物量生产的影响。在没有豆科作物

的情况下, 11 年后植物多样性依然能够增加土壤碳、氮的储量(第 2 章)。8 个物种的混作比这 8 个物种的单作平均增加了 18%和 16%的土壤碳、氮储量。增加的土壤碳、氮储量是由于植物生产力的提高从而增加了土壤碳投入和氮存留。除生物多样性以外, 一个非禾本的草本植物(车前菊)的存在, 也增加了土壤碳储量。由于多物种的植物群落包含这个物种的几率更大, 因此这个物种的存在也可能贡献了多物种体系下更高的土壤碳储量。

一些研究表明植物多样性加速了有机质的分解。这可能部分或者全部抵消物种多样性通过提高土壤碳投入来增加土壤碳固定的优势。从本研究使用的这个 11 年的定位试验地取土, 然后将土在实验室培养了 145 天去测定物种多样性对于土壤有机碳分解的影响。结果表明植物多样性提高了土壤有机质分解的相对速率(第 2 章)。这种速率的提高主要由于生物量生产的增加所引起。植物多样性可以通过改变有机质的质量或者改变分解的微环境(包括生物和非生物因素)来影响有机质的分解。因此, 很有可能的是, 增加的植物生产力(也就是更多的新鲜有机质的投入)提高了易分解的土壤碳在土壤整个碳库的比例, 进而减少了土壤有机质的抗分解性, 最终加速了土壤有机质的分解速率。此外, 堆肥被添加到种植过不同物种数目的土壤中, 结果呈现了相似的分解速率(第 3 章)。这进一步确认了增加的有机质的相对分解速率与土壤有机质的质量相关, 而与土壤分解的微环境的变化无关。通过一个实验室培养的试验, 我进一步测定了植物多样性对于新鲜根系的分解的影响。结果表明植物多样性加快根系分解是通过影响土壤的微环境, 而不是通过改变根系的多样性。显而易见, 无论是土壤有机质还是新鲜根系, 加速的土壤分解导致的土壤碳的损失并没有超过生产力的提高所增加的土壤碳。

一些研究显示植物多样性对于生物量的增加随着时间加强。可能的解释包括多物种的群落通过加快物种的演替或减少土壤病菌对植物的损害来维持高的群落



生产力。在这个研究中，我提出如下科学假设：增加的土壤碳、氮储量可促进土壤碳、氮的循环，进而增加植物的生产力。通过测定土壤氮矿化速率，我发现其随着物种多样性的增加而增加。这与增加的土壤碳、氮储量的结果相结合，可以解释为什么物种多样性对植物生产力的增加在最后几年得到加强（第 2 章）。

对于农业系统可持续性的担忧激发了人们极大的兴趣去探索生态型的管理措施来实现粮食安全，同时减少环境影响。其中，作物多样性种植作为生态集约化农业的措施正在越来越多的被推广。例如，间作（指的是在一块地里同时种两种或多种作物）就是农田生态系统增加多样性的一种方式。间作通常可以增加作物地上部分的生产力。增加的生产力被归功于物种之间在时间上或空间上的补偿机制，或物种间的促进作用，或者是病虫害的减轻和杂草的减少。近来的研究暗示，间作也可能会增加作物地下部分的生产力，但目前尚未被证实。理解作物多样性对土壤碳固定和土壤肥力的影响对于强化生态型的可持续农业非常关键。轮作和覆盖作物对于土壤碳、氮固定的优势已经被广泛的证实。然而，间作对于土壤碳储量的长期影响尚缺乏研究。虽然有一些报道，但是由于其试验周期太短而不能证实是否间作可以增加土壤碳、氮的固定。

本研究使用了一个 7 年的间作定位试验（这是目前存在的持续最长的间作试验）来测定间作对于土壤碳、氮储量的影响。我们的结果首次证明间作分别增加了 4% 和 11% 的土壤碳、氮储量（第 4 章）。此外，两个周期为 1 年的田间试验一致的显示间作增加了谷物产量和地上部生物量。更为重要的是，间作也增加了根系生物量峰值，平均达 23%，这为间作体系能增加土壤碳固定提供了合理的解释（第 4 章）。我们的结果显示与蚕豆间作的体系（也就是玉米/蚕豆和小麦/蚕豆间作）比蚕豆轮作的体系减少了土壤的同位素氮 15 的丰度值。这表明间作体系氮储量的增加来自于生物固氮量的提高（第 4 章）。然而，小麦/玉米体系也

增加了土壤氮储量，这强调了其他机制贡献了氮在土壤中的存留。比如，间作小麦与玉米由于根系深度的差异以及生物时期的不同可以获取更多的氮。此外，作物物候学的差异也可以增加氮的再循环，因为早期生长的小麦的根系所矿化出来的氮可能被后期生长的玉米所利用。

我们的结果表明在连续种植7年后，间作比单作增加了23%的根系生物量，却只增加了4%的土壤碳储量。为了研究间作对于土壤有机质的分解，我将该试验地的土壤在实验室培养了112天，发现间作的确增加了土壤有机质的相对分解速率（第5章）。增加的速率被归功于间作改变了土壤有机质的质量，而不是改变了有机质的分解的微环境。进一步的实验室以及田间试验被用来探索间作是否可以影响新鲜根系的分解，以及这种影响是通过改变土壤分解的微环境还是改变根系的多样性来影响根系分解（第5章）。结果表明间作的影响依赖于培养条件和间作作物的组合。在实验室培养的条件下，玉米/小麦间作通过对土壤分解微环境的影响降低了根茬分解速率。相反，在田间情况下，玉米/蚕豆间作通过根系多样性的提高增加了根茬的分解速率。玉米/小麦体系与单作相比增加了作物氮的吸收，这可能减少了土壤氮的有效性，从而降低了根茬分解速率。玉米/蚕豆体系中根系分解速率的提高可能是由于氮含量高的蚕豆根茬矿化出的土壤氮，促进了氮含量低的玉米根茬的分解。

总之，植物物种多样性增加了包括土壤碳、氮储量、生物量生产以及土壤有机碳的分解在内的几个生态系统功能，这在多年生、氮限制的草地系统和一年生、氮富集的农田系统表现一致。两个体系中土壤碳储量的增加归功于生物量的提高带来的碳投入的增加，但部分增加被加速的土壤有机质分解所抵消。含豆科作物的间作体系提高的土壤氮储量主要来自增加的土壤氮投入，不含豆科作物的间作提高的土壤氮储量则归功于氮存留量的增加。模型模拟的结果表明间作有着与其他较常推荐的种植体系（比如轮作）相当的土壤碳固定潜力，对温室

气体减排有着程度虽小、但不可忽略的贡献。更重要的是，间作可以改善土壤肥力，有助于长期维持生态系统生产力。因此，间作作为一种可改善生态系统服务功能的管理措施，可以同时提高作物生产，改善土壤质量和减轻温室气体排放。进一步的研究可以探索植物多样性对于长期土壤有机质分解的影响以及系统研究物种多样性的草地和间作体系中包括反硝化过程在内的植物-土壤氮动态。这将有助于未来全面的评估植物物种多样性对于减缓温室气体排放的潜力。



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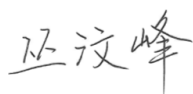
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Now, my PhD thesis is complete. And my scientific career will go on!

A handwritten signature in black ink, consisting of three characters: '文', '峰', and '丛' (Wenfeng Cong).

Wenfeng Cong

Wageningen

19 May 2014



## Publication list

### *Journal articles:*

- Wen-Feng Cong**, Jasper van Ruijven, Liesje Mommer, Gerlinde De Deyn, Frank Berendse and Ellis Hoffland. Plant species richness promotes soil carbon and nitrogen storage in grasslands without legumes. Accepted for publication in *Journal of Ecology*.
- Wen-Feng Cong**, Ellis Hoffland, Long Li, Johan Six, Jian-Hao Sun, Xing-Guo Bao, Fu-Suo Zhang and Wopke van der Werf. Learning from nature: ecological intensification through intercropping enhances sequestration of soil carbon and nitrogen. Under revision in *Global Change Biology*.
- Wen-Feng Cong**, Ellis Hoffland, Long Li, Bert H. Janssen, Wopke van der Werf. Crop diversification by intercropping affects decomposition of soil organic matter and root litter. (Submitted)
- Wen-Feng Cong**, Jasper van Ruijven, Wopke van der Werf, Liesje Mommer, Frank Berendse and Ellis Hoffland. Plant species richness accelerates decomposition of root litter through a soil-mediate effect. (Submitted)
- Rob W Brooker, Alison E Bennett, **Wen-Feng Cong**, Tim J Daniel, Tim S George, Cathy Hawes, Pete PM Iannetta, Hamlyn G Jones, Alison J Karley, Haigang Li, Long Li, Blair M McKenzie, Robin J Pakeman, Eric Paterson, Christian Schöb, Jianbo Shen, Geoff Squire, Christine Watson, Chaochun Zhang, Junling Zhang, Paul D Hallett, Philip J White. Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. Under revision in *New Phytologist*.

### *Conference proceedings:*

- Wen-Feng Cong**, Wopke van der Werf, Ellis Hoffland, Holger Meinke, Long Li, Fu-Suo Zhang. Soil carbon sequestration in intercropping agroecosystems. Proceeding of the Conference “Soil Science in a Changing World”, 18-22 September, 2011.

**Wen-Feng Cong**, Jasper van Ruijven, Liesje Mommer, Gerlinde De Deyn, Frank Berendse and Ellis Hoffland. Plant species richness promotes soil carbon and nitrogen storage in grasslands without legumes. Proceeding of the Netherlands Annual Ecology Meeting 2014, 11-12 February, 2014.

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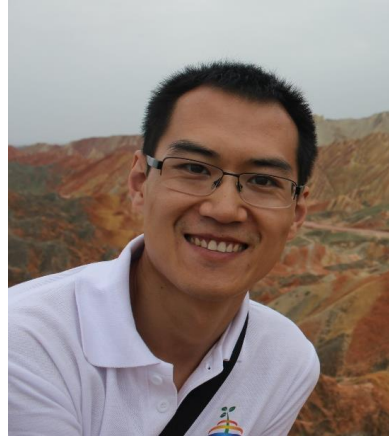
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## *Curriculum vitae*

Wenfeng Cong was born in the beautiful coastal city of Weihai, in Shandong Province, China on the 1<sup>st</sup> of March, 1983. He received his primary and secondary education in his home town. In 2003, he left for Haerbin, a city in northeast China to study at Northeast Agricultural University. During his university life, he enjoyed participating in and organizing a variety of student activities. In 2006, he did his internship at Jiansanjiang Research Farm, studying the effect of site-specific nitrogen management on grain yield of irrigated rice. He finished his Bachelor of Sciences degree (Agronomy) in 2007. From then on, he continued his interest in agricultural researches by pursuing a Master of Science Degree in Agronomy at China Agricultural University under the supervision of Prof. Fusuo Zhang. For his master thesis, he explored the responses of rhizosphere microbial biomass to intercropping, as influenced by nitrogen and phosphorus fertilization. After graduation in late 2009, he successfully applied for a scholarship from the China Scholarship Council to study abroad. On the occasion of Sino-Dutch collaboration on intercropping, he decided to go to the Netherlands and enrolled in the PhD programme of Wageningen University in March 2010. During his PhD study, he worked on the thesis entitled “On Soil Organic Matter Dynamics in Species-Diverse Grasslands and Intercrop Systems” at the Crop and Weed Ecology Group of the Centre for Crop Systems Analysis. He completed his PhD study in March 2014.





## PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



### Review of literature (4.5 ECTS)

- Can intercropping enhance soil organic C storage? (2010)

### Writing of project proposal (4.5 ECTS)

- Soil organic carbon dynamics in intercropping systems (2011)

### Post-graduate courses (5.6 ECTS)

- Soil ecology: taking global issues underground; SENSE (2010)
- The art of modelling; SENSE (2010)
- The legume-rhizobium symbiosis from molecules to farmers' fields; WUR (2010)
- Mixed linear model; PE&RC (2012)

### Laboratory training and working visits (1.2 ECTS)

- Sampling protocols for ecosystem research; VU University, Amsterdam (2013)

### Invited review of (unpublished) journal manuscript (2 ECTS)

- Plant and Soil: long-term fertilisation and crop rotation effects on soil microbial biomass and microbial residues in semi-arid tropics of India (2012)
- Agroforestry Systems: research on carbon stock of protected forests on China's plains (2012)

### Deficiency, refresh, brush-up courses (3 ECTS)

- Designing sustainable cropping systems (2010)
- Systems analysis, simulation and systems management (2010)
- Advanced statistics (2010)
- Ecological modelling in R (2012)

### Competence strengthening / skills courses (3.6 ECTS)

- PhD Competence assessment; WGS (2010)
- Project and time management; WASS (2012)
- Scientific writing; WGS (2012)

### PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Day (2010 & 2011)
- PE&RC Weekend (2010 & 2013)

### Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- Lunch seminar, Centre for Crop Systems Analysis & Soil Quality Group (2010-2013)
- Climate change – Soil-water-atmosphere Interactions- “CSI Wageningen” (2010-2013)

### International symposia, workshops and conferences (6 ECTS)

- Wageningen Conference on Applied Soil Science: soil science in a changing world (2011)
- Workshop: mechanisms behind high yield and efficient resource utilization in intercropping; Beijing, China (2014)
- Netherlands Annual Ecology Meeting; Lunteren, the Netherlands (2014)

### Lecturing / supervision of practical's / tutorials (0.9 ECTS)

- Soil-Plant-Interactions (2012)
- Soil-Plant-Interactions (2014)

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