Soil greenhouse gas (N₂O, CO₂ and CH₄) fluxes from cropland agroforestry and monoculture systems

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Guodong Shao

Born in Shandong, China

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1st Referee: Prof. Dr. Edzo Veldkamp

2nd Referee: Prof. Dr. Alexander Knohl

Supervisors: Prof. Dr. Edzo Veldkamp, Dr. Marife D. Corre, and Dr. Guntars Martinson

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Summary

Conventional agriculture is the dominant contributor to negative environmental impacts such as the growth in global greenhouse gas (GHG) emissions, and the challenges are likely to increase with the increasing global food demand as well as the agricultural expansion. Agroforestry is a sustainable management practice with strong potential to provide ecosystem services and environmental benefits through increasing carbon sequestration, nutrient availability, water use efficiency and biodiversity, and reducing soil erosion and nitrogen losses. Therefore, the establishment of agroforestry practices offers an opportunity to reduce GHG emissions. Previous studies have showed the effects of agroforestry on soil nitrous oxide (N₂O), carbon dioxide (CO₂), and methane (CH₄) fluxes in many parts of the world. In temperate Europe, the information on the GHG mitigation potential of agroforestry compared to cropland monoculture is still unclear. The present thesis consists of two studies, which was designed to explore whether the conversion of cropland monoculture to agroforestry systems reduces trace gases N₂O, CO₂, and CH₄ emissions from the soil. The study was carried out at three sites varied with soil types in Germany. Each site had adjacent alley cropping agroforestry and cropland monoculture systems and the trees in agroforestry system were planted 1 to 11 years prior to this research. We measured soil N₂O, CO₂, and CH₄ fluxes monthly using vented static chambers at the three sites from March 2018 to January 2020. On each day of gas sampling, soil temperature, water-filled pore space and extractable mineral nitrogen (N) were measured in the top 5 cm.

The objective of our first study was to quantify the spatial-temporal dynamics of soil N₂O fluxes from cropland agroforestry and monoculture systems, following different crop rotations and fertilization rates. The pattern of soil N₂O fluxes were predominantly controlled by soil mineral N in both agroforestry and monoculture systems. The positive relationship between water-filled pore space with soil N₂O fluxes during the cropping seasons, indicating soil moisture acts as a limiting factor under N-sufficient conditions. The entire agroforestry systems tended to reduce soil N₂O emissions by 9% to 56% compared to monocultures, during the corn phase of the rotation that had typically high fertilization rates. The lowest soil N₂O emissions

in the unfertilized tree rows (occupied 20% of the agroforestry area) represent a potential for mitigating N_2O emissions from croplands.

The objective of our second study was to investigate the changes in soil CO₂ and CH₄ fluxes after conversion from cropland monoculture to alley cropping agroforestry systems. Our results showed that seasonal variations of soil CO₂ and CH₄ fluxes were strongly regulated by soil temperature and moisture, and the spatial variations were mainly controlled by texture. The establishment of agroforestry systems had no effect on reducing soil CO₂ emissions, possibly because there was no significant difference in soil temperature between management systems. Annual soil CH₄ uptake in the agroforestry systems was increased by up to 300% compared to monocultures, which may be related to the regulation of trees on soil moisture in agroforestry systems.

The present research provides the first insight into the systematic comparison of soil N₂O, CO₂ and CH₄ fluxes from cropland agroforestry and monoculture systems, and it provides a unique dataset for estimating the net balance of carbon emissions after conversion of cropland monoculture to alley cropping agroforestry system in temperate regions. Although soil CO₂ emissions showed no differences between management systems, the total annual soil emissions of non-CO₂ GHG from agroforestry systems were reduced by 0.22 Mg CO₂ eq ha⁻¹ compared to the monocultures. Considering the driving function of soil moisture and mineral N on soil GHG fluxes from cropland agroforestry and monoculture systems, our findings suggest that improved system management (e.g. optimal adjustments of the areal coverages between tree and crop rows) and optimized fertilizer input will enhance the potential of cropland agroforestry for mitigating N₂O emissions and increasing CH₄ uptake and C sequestration in the long run.

Zusammenfassung

Konventionelle Landwirtschaft trägt mit am stärksten zum Anstieg der globalen Treibhausgasemissionen und die Herausforderungen werden durch den Anstieg der globalen Nahrungsmittelnachfrage und der landwirtschaftlichen genutzten Fläche weiter zunehmen. Die Agroforstwirtschaft ist eine nachhaltige Bewirtschaftungsform in der Landwirtschaft. Sie weist großes Potenzial auf, Ökosystemdienstleistungen und die Umweltbedingungen durch Erhöhung der Kohlenstoffspeicherung, Nährstoffverfügbarkeit, Wassernutzungseffizienz und Biodiversität und durch Reduktion von Bodenerosion und Stickstoffverlusten zu verbessen. Daher ermöglicht die Etablierung agroforstwirtschaftlicher Praktiken, Treibhausgasemissionen zu reduzieren. Studien haben gezeigt, dass die Agroforstwirtschaft einen Einfluss auf die Treibhausgasflüsse von Stickstoffdioxid (N2O), Kohlenstoffdioxid (CO2) und Methan (CH4) in vielen Regionen der Erde haben. Im gemäßigten Europa gibt es allerdings nur wenige Informationen über das Treibhausgaseinsparpotenzial der Agroforstwirtschaft im Vergleich zur konventionellen Landwirtschaft. Anhand zweier Studien untersucht die vorliegende Promotionsarbeit den Einfluss der Umwandlung landwirtschaftlicher Monokulturen zu Agroforstkulturen auf die Bodentreibhausgasflüsse und ob die Umwandlung zu einer Reduktion von N2O, CO2 und CH4 führt. Die Studien wurden an drei Standorten in Deutschland mit unterschiedlichen Bodentypen durchgeführt. Jeder der Standorte bestand aus einer landwirtschaftlichen Monokultur und einer benachbarten "Alley Cropping" - Agroforstkultur. Die Baumreihen in den "Alley Cropping" Agroforstkulturen waren 1-11 Jahre alt. Im Rahmen der Untersuchungen wurden auf allen Untersuchungsflächen N2O, CO2 und CH4 Bodenflüsse mithilfe belüfteter, statischer Luftkammern und der Gaschromatographie von März 2018 bis Januar 2020 gemessen. Des Weiteren wurden bei jeder Beprobung auch Bodentemperatur, wassergefülltes Porenvolumen und extrahierbarer mineralischer Stickstoff in den ersten 5 cm des Oberbodens gemessen.

Das Ziel der ersten Studie der Promotionsarbeit war die Quantifizierung räumlichzeitlicher Dynamiken der Boden-N₂O Flüsse sowohl in den "Alley Cropping" – Agroforstkulturen als auch in den landwirtschaftlichen Monokulturen unter Berücksichtigung verschiedener Nutzpflanzenzyklen und Düngeraten. Das Muster der Boden-N₂O-Flüsse wurde sowohl in Agroforst- als auch in Monokultursystemen überwiegend durch Bodenmineral-N gesteuert. Die positive Beziehung zwischen wassergefüllten Porenräumen und Boden-N2O-Flüssen während der Erntesaison, was darauf hindeutet, dass die Bodenfeuchtigkeit unter Nausreichenden Bedingungen als limitierender Faktor wirkt. Die gesamten Agroforstsysteme tendierten dazu, die N₂O-Emissionen des Bodens um 9 bis 56 % im Vergleich zu Monokulturen während der Mais-Rotationsphase mit typischerweise hohen Düngeraten zu reduzieren. Die niedrigsten N₂O-Emissionen des Bodens in den ungedüngten Baumreihen (ca. 20% der Gesamt-Agroforstkulturfläche) stellen ein Potenzial zur Minderung der N₂O-Emissionen aus Ackerland dar.

Das Ziel der zweiten Studie der Promotionsarbeit war Identifikation von Veränderungen der Boden-CO₂ und -CH₄ Flüsse infolge der Transformation von Monokulturen zu "Alley Cropping"-Agroforstkulturen. Die saisonalen Änderungen der Boden-CO₂ und -CH₄ Flüsse wurden durch die Bodentemperatur und Bodenfeuchte und die räumlichen Änderungen durch die Bodentextur stark reguliert. Die Boden-CO₂ Emissionen unterschieden sich nicht zwischen den verschiedenen Nutzungssystemen. Unter Berücksichtigung aller Standorte erhöhten Agroforstkulturen die CH₄-Aufnahmerate um bis zu 300% im Vergleich zu den Monokulturen. Dies lag wahrscheinlich an der regulierenden Wirkung der Baumvegetation auf die Bodenfeuchte.

Insgesamt liefert die Promotionsarbeit den ersten systematischen Vergleich von Boden-CO₂, -N₂O und -CH₄ Flüssen zwischen Agroforst- und Monokulturen und liefert damit eine einzigartige Datengrundlage, um die Nettobilanz von Kohlenstoffemissionen bei der Transformation von Mono- zu Agroforstkulturen in gemäßigten Klimazonen abzuschätzen. Obwohl die Transformation keinen signifikanten Einfluss auf die CO₂ Emissionen hatte, führten die Agroforstkulturen zu einer Reduktion der Nicht-CO₂ Treibhausgasemissionen um ca. 0.22 Mg CO₂ eq ha⁻¹. Daher zeigen unsere Ergebnisse, dass ein angepasstes Düngungsmanagement und eine effiziente Düngemittelgabe das Potenzial von Agroforstkulturen, Boden-N₂O Emissionen zu reduzieren und Boden-CH₄ Aufnahmeraten zu erhöhen und die Kohlenstoffspeicherung zu steigern, langfristig erhöhen kann.

Chapter 1

General Introduction

1.1. Agroforestry and its environmental impacts

Global demand for agricultural production is increasing with continuously growing population (Mauser et al., 2015). During the past three decades, crop production has increased markedly from intensively managed agricultural systems, with the excessive use of synthetic fertilizers, pesticides, and herbicides (Pretty, 2018). However, agricultural intensification has already been a dominant cause of numerous global environmental impacts including land degradation and erosion, freshwater pollution, biodiversity loss and climate change (Dirzo and Raven, 2003; Foley et al., 2005; Pretty, 2018). Currently, agricultural activities are responsible for approximately 11% of global anthropogenic greenhouse gas (GHG) emissions (IPCC, 2014). These environmental challenges are likely to increase as the increasing global food demand (Bajželj et al., 2014) as well as the agricultural expansion (Tilman et al., 2011). Thus, there is widespread concern about calling for more sustainable agriculture that can achieve global food security while reducing environmental impacts (Foley et al., 2011; Tilman et al., 2011; Bajželj et al., 2014; Pretty, 2018).

Agroforestry, is an agricultural management practice that integrates trees with crops and/or animals simultaneously on the same land (Brown et al., 2018). Modern agroforestry systems are generally classified by FAO as three types include agrisilvicultural system (trees and/or shrubs integrated with cropping systems), silvopastoral system (trees and/or shrubs integrated with livestock), and agrosylvopastoral system (trees integrated with both crops and livestock). In the last decades, agroforestry practices have received increasing attention globally for their advantage of providing numerous ecosystem services and environmental benefits that may be lacking in conventional agricultural systems (Jose, 2009; Quinkenstein et al., 2009; Tsonkova et al., 2012). Agroforestry systems can provide ecological benefits through increasing carbon (C) sequestration (Kim et al., 2016; Peichl et al., 2006), nutrient availability (Pardon et al., 2017), water use efficiency (Schwendenmann et al., 2010) and biodiversity (Banerjee et al., 2016; Beule and Karlovsky, 2021), and reducing soil erosion and N losses (Andrianarisoa et al., 2016; Wolz et al., 2018). Agroforestry therefore, is widely promoted due to its strong potential for climate change mitigation and adaptation (Quinkenstein et al., 2009; Zomer et al., 2016; Brown et al., 2018; Wolz et al., 2018).

Compared with conventional monoculture croplands, agroforestry systems have positive effects on soil quality and water regulation (Jose, 2009; Dollinger and Jose, 2018). Numerous studies have reported that agroforestry systems can increase soil organic carbon (SOC) content both in tropics and temperate regions of the world. Amadi et al. (2016) demonstrated that the establishment of shelterbelts can increase SOC storage by 27% compared to the adjacent cropping areas in Canada, mainly attributed to the enhanced fine root turnover and continuous tree-litter input to the soil. Similarly, Zake et al. (2015) observed significantly higher total soil organic matter (SOM) and total nitrogen (N) in the banana-coffee agroforestry farming systems than the banana monoculture in Central Uganda. In addition, the potential for C sequestration may depend on the ages of trees within agroforestry systems (Kim et al., 2016). For example, a poplar-based agroforestry system was reported to have 2.9-4.8 Mg ha⁻¹ higher SOC compared to monoculture cropland in central Punjab of Northwest India, and the improvement increased with tree age of 1-6 years (Gupta et al., 2009). Pardon et al. (2017) assessed the differences in SOC and nutrient status from young (< 5 years) and middle-aged to mature (15-47 years) agroforestry systems and adjacent arable fields in Belgium, they found that SOC and soil nutrient concentrations of N, P, K, Mg, and Na were only increased in the middle-aged to mature agroforestry systems compared to the boundary planted fields, and the increase of these soil variables was strongly related to the distance from the tree row.

It has been recognized that introducing trees into cropping systems plays an important role in regulating soil water availability (Quinkenstein et al., 2009; Tsonkova et al., 2012). For example, trees in agroforestry system can decrease soil evaporation by reducing wind speed (Swieter et al., 2019), and consequently, water losses in the system (Lin, 2010). However, nutrient competition may also occur between trees and crops in agroforestry systems (Jose et

al., 2000; Zamora et al., 2009) because tree roots can extract soil N and water sources from the crop row at a distance up to two times the height of the trees (Allen et al., 2004). In addition to the beneficial impacts on soil nutrient and water availability, agroforestry also contributes to the improvement of soil microbial abundance and diversity (Jose, 2012; Dollinger and Jose, 2018). Banerjee et al. (2016) illustrated that trees in agroforestry systems enhanced soil bacterial abundance and species richness, and the promotion is possible to be predicted to some extent. Beule et al. (2020) assessed different soil microbial communities that were involved in N₂ fixation, nitrification, and denitrification processes from three alley cropping agroforestry systems in Germany, they found poplar trees in agroforestry systems increased several soil microbial abundance and N-cycling genes compared to the adjacent crop rows and conventional monocultures. Similarly, Beuschel et al. (2019) reported soil microbial biomass and enzyme activities in the tree row in the 5-cm soil depth were significantly increased after the implementation of trees in arable systems within 5–8 years.

Overall, agroforestry systems present great potential for delivering important ecosystem functions through their positive effects on soil physical, chemical, and biological properties. These benefits also make agroforestry provide opportunities not only to improve soil health but also to mitigate GHG emissions from agricultural soils.

1.2. Greenhouse gas fluxes from soil

Soils can act as both sources and sinks of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), which are the three most important GHGs that contribute to global warming (Solomon et al., 2007). Of the total global anthropogenic GHG emissions, 38% are estimated to originate from the land use sector of agriculture, forestry, and other land use (AFOLU) (IPCC, 2014). Moreover, agricultural soils have been identified as one of the main GHG source categories within the agricultural sector (Lokupitiya & Paustian, 2006). In soils, the production and consumption processes of the three GHGs largely depend on a variety of biotic and abiotic factors (Oertel et al., 2016).

CO₂ is the most dominant GHG produced by the burning of fossil fuels, industrial

production and land use change, accounting for around three-quarters of the total global GHG emissions (Olivier and Peters, 2019). Since 2010, the atmospheric CO₂ concentration increases at an average rate of 0.6 percent per year. Soil respiration represents the second-largest C flux between terrestrial ecosystems and atmosphere (Hanson et al., 2000). Even small changes in soil respiration are likely to affect CO₂ concentration in the atmosphere and further impact global C cycle (Bahn et al., 2009). Soil CO₂ fluxes are the result of respiration processes from soil organic matter (SOM) decomposition by soil microbes (heterotrophic respiration) and roots (autotrophic respiration) (Hanson et al., 2000). These processes are primarily influenced by soil temperature and moisture (Davidson et al., 2006; Meyer et al., 2018). It has been well illustrated that soil CO₂ emissions are positively correlated with temperature in both cropping and forest systems (Gauder et al., 2012; Luo et al., 2012; Wordell-Dietrich et al., 2020). Soil CO₂ emissions generally exhibit a parabolic relationship with soil moisture, with emissions increased under favorable moisture conditions and decreased when soils are very wet that may limit gas diffusion and/or CO₂ production (Koehler et al., 2009; Franzluebbers et al., 2017; Tchiofo Lontsi et al., 2020). Earlier studies suggest that the spatial variability of soil CO₂ emissions can also be influenced by texture (Sotta et al., 2006; Hassler et al., 2015), substrate availability (Gershenson et al., 2009), vegetation type (Raich & Tufekcioglu, 2000), and landuse change (Edzo Veldkamp et al., 2020).

CH4 is the second most important greenhouse gas contributor to climate change after CO₂, with a global warming potential 28–34 times greater than CO₂ on a 100-year time scale (IPCC, 2014). Global atmospheric concentration of CH4 has increased from a pre-industrial level of 720 ppb to 1860 ppb in 2018 (Jackson et al., 2019). Globally, wetlands make up for the largest natural source of CH4 to the atmosphere (Kirschke et al., 2013; Poulter et al., 2017), while the upland soils, are generally recognized as net sinks for atmospheric CH4 (Dutaur & Verchot, 2007). Soils act as a source or sink of CH4 depending on the balance between the production of CH4 by methanogenic microorganisms under anaerobic conditions and oxidation by methanotrophic microorganisms under well-aerated conditions (Le Mer & Roger, 2001). Thus, soil CH4 fluxes are strongly determined by environmental parameters that can influence gas diffusion and soil microbial activity (Dobbie and Smith, 1996;Veldkamp et al., 2013;

Gatica et al., 2020). The primary controlling factors of soil CH₄ fluxes are soil moisture, temperature and texture (Dutaur and Verchot, 2007; Gauder et al., 2012; Veldkamp et al., 2013; Walter et al., 2015; Matson et al., 2017). In addition, CH₄ production and consumption in soils could also be influenced by N availability (Veldkamp et al., 2013; Martinson et al., 2021) and pH (Borken et al., 2003).

N₂O is the third-largest contributor of long-lived GHG emissions to the atmosphere (IPCC, 2014) and is the main contributor to stratospheric ozone depletion (Ravishankara et al., 2009), with a global warming potential 265–298 times greater than CO₂ at a 100-year time horizon (IPCC, 2014). The atmospheric concentration of N₂O has increased steadily at a rate of ~0.73 ppb yr⁻¹ over the last 30 years (IPCC, 2014). Agricultural soils contribute to the largest source of global anthropogenic N₂O emissions (Ciais et al., 2013), largely due to the increasing use of reactive nitrogen (N) based fertilizers and manure on agricultural land (Eric A. Davidson, 2009). As the increasing global demand for agricultural food production (Mauser et al., 2015), N₂O emissions from agriculture are likely to continue increasing in coming decades (Tilman et al., 2011; Bajželj et al., 2014). The production of N₂O is mainly caused by a combination of microbial nitrification and denitrification processes. In nitrification, ammonium (NH4⁺) as the substrate, is oxidized to nitrite (NO2⁻) and nitrate (NO3⁻) under aerobic conditions. In denitrification, NO3⁻ is used as an electron acceptor under anaerobic conditions and reduced to nitrogen (N₂), while N₂O is produced as a by-product (Butterbach-Bahl et al., 2013). Therefore, soil moisture condition and N availability play a vital role in driving the production and release of N₂O from the soil. In addition, previous studies have also demonstrated the regulation of temperature (Roelandt et al., 2005), pH (Wang et al., 2018), and land-use changes on soil N₂O fluxes (Díaz-Pinés et al., 2017; Hassler et al., 2017).

1.3. Effects of agroforestry on soil greenhouse gas fluxes

Agroforestry system has been widely investigated for its potential in mitigating GHG emissions from agriculture (Dixon, 1995; Kim et al., 2016). Changes in soil CO₂, CH₄ and N₂O fluxes after the establishment of agroforestry systems are largely due to the changes in soil variables (Peichl et al., 2006; Amadi et al., 2016; Kim et al., 2016). In agroforestry systems, soil CO₂

emissions can be increased under trees or adjacent cropped fields, which probably contribute to the increased root respiration of trees during growing periods and enhanced soil organic carbon decomposition by tree litter input (Amadi et al., 2016). However, introducing trees into croplands may also maintain (Medinski et al., 2015) or decrease (Franzluebbers et al., 2017) soil CO₂ emissions depending on the stages of tree growth. In addition to the influence on soil CO₂ fluxes, agroforestry systems play a role in regulating annual C budget by increasing both above- and belowground biomass stocks and enhancing C sequestration (Jose, 2009; Kim et al., 2016). Estimated net C balance for the agroforestry and sole cropping systems indicates the potential of agroforestry systems to act as C sink and to reduce atmospheric CO₂ concentration (Peichl et al., 2006). Changes in soil CH₄ fluxes are mainly associated with land-use induced changes of soil moisture and bulk density, which directly influence gas diffusion and thus CH4 uptake (Amadi et al., 2017). Agroforestry systems can strongly reduce soil N₂O emissions compared to monoculture croplands, which may be related to the lacking of N fertilizer application under the trees (Franzluebbers et al., 2017), and can also contribute to the cooler temperature in agroforestry systems (Quinkenstein et al., 2009). Due to the reduction of fertilizer input and higher N use efficiency, agroforestry systems potentially decrease nitrification rates, which consequently mitigate N₂O emissions (Thevathasan & Gordon, 2004).

1.4. Agroforestry in temperate Europe

In Europe, agroforestry systems are gaining increasing interest as they present a large potential for solving important environmental problems (Nerlich et al., 2013). In comparison to conventional agriculture, modern agroforestry systems seem to be a promising alternative in current farming practices. The implementation of agroforestry has been promoted by the European Common Agricultural Policy (CAP) through supporting farmers to develop agroforestry practices on arable land, permanent grassland, and permanent crops (Mosquera-Losada et al., 2017). Alley cropping system, is one of the novel agroforestry practices that combine both agriculture and short rotation coppices (SRC) for bioenergy production, as integrates trees or shrubs into conventional croplands on the same field (Tsonkova et al., 2012; Wolz et al., 2018). The woody components in SRC mainly include fast-growing tree species

like poplar and willow that have been recognized for producing high biomass yields and reducing management costs (Bredemeier et al., 2015). In addition, the SRC plantations are generally not fertilized due to their lower fertilization demands compared to other bioenergy crops (Tsonkova et al., 2012; Karp & Shield, 2008), especially when SRCs are established on former cropland (Schmidt-Walter & Lamersdorf, 2012). Thus, the SRCs in alley cropping agroforestry system may help to provide the potential for mitigating greenhouse gas emissions (Díaz-Pinés et al., 2017; Horemans et al., 2019).

In Germany, few experimental alley cropping agroforestry systems have been cultivated in the last decade, and the changes in crop yield (Swieter et al., 2019), biomass production (Böhm et al., 2014; Lamerre et al., 2015), nutrient response efficiency (Schmidt et al., 2021), and soil microbial communities (Beuschel et al., 2019; Beule et al., 2020; Beuschel et al., 2020; Beule and Karlovsky, 2021) have been widely studied after the establishment. To date, there is only one study that has focused on the potential of alley cropping agroforestry systems for mitigation of CO₂ emissions from soil, based on a seven months measurement period (Medinski et al., 2015). The present research was carried out at three sites with varied soil types in Germany. Each site had adjacent alley cropping agroforestry and cropland monoculture systems. The alley cropping agroforestry systems combined cropland and hybrid poplar SRC for bioenergy production, and the tree rows are unfertilized (Schmidt et al., 2021). The conversion from cropland monocultures to agroforestry systems occurred 1 to 11 years prior to this research.

1.5. Aims and hypotheses

The present research consists of two studies that aimed to investigate the effects of converting cropland monoculture to alley cropping agroforestry system on soil greenhouse gas (N₂O, CO₂ and CH₄) fluxes, based on a two-year field measurement following different crop rotations and fertilization rates at three sites on different soils in Germany. These studies provide the first systematic comparison of soil greenhouse gas fluxes between cropland agroforestry and monoculture systems, which the information is lacking in temperate Europe.

The objectives of our first study were (1) to quantify the spatial-temporal dynamics of soil N₂O fluxes, and (2) to determine their controlling factors in cropland agroforestry and monoculture systems. We hypothesized that soil N₂O emission from unfertilized tree row will be lower than that from crop row. Therefore, when integrate agroforestry as a whole, soil N₂O emissions from agroforestry will be lower than from cropland monoculture systems.

The objectives of our second study were to (1) assess the changes in soil CO₂ and CH₄ fluxes after conversion of cropland monoculture to alley cropping agroforestry system, and (2) determine the temporal and spatial controls of soil CO₂ and CH₄ fluxes. We hypothesized that (1) alley cropping agroforestry systems will have higher soil CO₂ emissions and CH₄ uptake than cropland monocultures, and (2) the temporal pattern of soil CO₂ and CH₄ fluxes will be regulated by soil moisture and temperature, soil CH₄ fluxes will be increased with increasing mineral N availability; the spatial patterns of soil CO₂ and CH₄ fluxes will be regulated by soil texture.

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Chapter 2

Impacts of monoculture cropland to alley cropping agroforestry conversion on soil N₂O emissions

Guodong Shao, Guntars Martinson, Jie Luo, Xenia Bischel, Dan Niu, Marife D. Corre, Edzo Veldkamp

Soil Science of Tropical and Subtropical Ecosystems, Faculty of Forest Sciences and Forest Ecology, University of Goettingen, Goettingen, Germany

Abstract

Monoculture croplands are a major source of global anthropogenic emissions of nitrous oxide (N₂O), a potent greenhouse gas that contributes to ozone depletion. Agroforestry has the potential to reduce N₂O emissions. Presently, there is no systematic comparison of soil N₂O emissions between cropland agroforestry and monoculture systems in Central Europe. Therefore, we investigated the effects of converting monoculture cropland to alley cropping agroforestry system on soil N2O fluxes at three sites (each site has paired agroforestry and monoculture) in Germany, where agroforestry combined crop rows and poplar short rotation coppice (SRC). We measured soil N₂O fluxes monthly over 2 years (March 2018–January 2020) using static vented chambers. Annual soil N2O emissions from the entire agroforestry and monocultures ranged from 0.21 to 2.73 kg N ha⁻¹ yr⁻¹ and 0.34 to 3.00 kg N ha⁻¹ yr⁻¹, respectively. During the corn phase of the rotation that had typically high fertilization rates, agroforestry reduced soil N₂O emissions by 9% to 56% compared to monocultures. This was caused by low soil N₂O emissions from the unfertilized agroforestry tree rows. Soil N₂O fluxes were predominantly controlled by soil mineral N in both agroforestry and monoculture systems. Our findings suggest that improved system management (e.g. optimal adjustments of the areal coverages between the tree and crop rows) and optimized fertilizer input will enhance the potential of agroforestry for mitigating N₂O emissions.

Keywords: nitrous oxide, cropland agroforestry, cropland monoculture, short-rotation coppice, soil greenhouse gas flux, nitrogen fertilization, crop type

2.1. Introduction

Nitrous oxide (N₂O) is the second most important non-carbon dioxide (CO₂) greenhouse gas (IPCC, 2014) and is the main contributor to stratospheric ozone depletion (Ravishankara et al., 2009). The atmospheric concentration of N₂O has increased steadily at a rate of ~ 0.73 ppb yr⁻¹ over the last 30 years (IPCC, 2014). Agriculture is the largest source of global anthropogenic N₂O emissions (Ciais et al., 2013), largely due to the increasing use of reactive nitrogen (N) based fertilizers and manure on agricultural land (Davidson, 2009). As the global demand for agricultural food production is increasing (Mauser et al., 2015), N₂O emissions from agriculture will increase as well (Tilman et al., 2011; Bajželj et al., 2014).

Agroforestry is an agricultural management practice that integrates trees with crops and/or animals simultaneously on the same land (Brown et al., 2018) and is widely promoted due to its strong potential for climate change mitigation and adaptation (Quinkenstein et al., 2009; Zomer et al., 2016; Brown et al., 2018; Wolz et al., 2018a). It can provide numerous ecosystem services and environmental benefits through increasing soil water use efficiency (Schwendenmann et al., 2010b), nutrient availability (Pardon et al., 2017), carbon (C) sequestration (Peichl et al., 2006), and biodiversity (Banerjee et al., 2016; Beule and Karlovsky, 2021), and reducing soil erosion and N losses (Wolz et al., 2018a). Soil N₂O emissions are expected to decrease following tree integration into monoculture croplands (Díaz-Pinés et al., 2017; Luo et al., 2022). However, there are only few studies about soil N₂O emissions from agroforestry systems in temperate regions. Beaudette et al. (2010) found that soil N₂O emissions from conventional monocropping systems were three times higher than hybrid poplar-based alley cropping agroforestry systems in Eastern Canada. Similarly, Franzluebbers et al. (2017) found that soil N₂O emissions were strongly reduced by the establishment of alley cropping system in southeastern USA because N fertilizer was not applied under trees. Due to the reduction of fertilizer input and higher N use efficiency, tree-based intercropping systems potentially decreased nitrification rates, which consequently mitigated N₂O emissions (Thevathasan & Gordon, 2004). However, in a fruit and nut trees-based alley cropping system, Wolz et al. (2018b) demonstrated that soil N₂O emissions were quickly reduced in comparison with the adjacent maize-soybean rotation agriculture, even though each system received the

same N fertilization rates.

The production of N₂O is mainly caused by a combination of microbial nitrification and denitrification processes. In nitrification, ammonium (NH4⁺) as the substrate, is oxidized to nitrite (NO₂⁻) and nitrate (NO₃⁻) under aerobic conditions. In denitrification, NO₃⁻ is used as an electron acceptor under anaerobic conditions and reduced to nitrogen (N₂), while N₂O is produced as a by-product (Butterbach-Bahl et al., 2013). Therefore, soil moisture condition and N availability control soil N2O fluxes (Davidson et al., 2000). Cropland soil environmental conditions are strongly influenced by trees in agroforestry systems (Amadi et al., 2017; Franzluebbers et al., 2017). Few studies have found increased competition for nutrients between trees and crops within agroforestry systems compared to cropland monoculture systems (Jose et al., 2000; Zamora et al., 2009). Allen et al. (2004) reported that tree roots extract soil N and water from the crop row at a distance up to two-times the height of the trees, which may reduce N₂O emissions by decreasing rates of denitrification (Beaudette et al., 2010). Ashraf et al. (2019) reported that by introducing oil palm trees in a former cropland monoculture system soil microbial abundance and enzyme activities may increase (Beuschel et al., 2019). However, the impacts of cropland monoculture to agroforestry systems conversion on soil water content and microbial activities may change over years after establishment (Beuschel et al., 2019; Clivot et al., 2019).

Alley cropping system, is one of the novel agroforestry practices that combines both agriculture and short rotation coppices (SRC) for bioenergy production, as it integrates trees or shrubs into conventional croplands on the same field (Tsonkova et al., 2012; Wolz et al., 2018a). The woody components in SRC mainly include fast-growing tree species like poplar and willow that has been recognized for producing high biomass yields and reducing management costs (Bredemeier et al., 2015). In addition, the SRC plantations are generally not fertilized due to their lower fertilization demands compared to other bioenergy crops (Karp & Shield, 2008), especially when SRCs are established on former croplands (Schmidt-Walter & Lamersdorf, 2012). Hence, SRCs in alley cropping agroforestry may reduce soil N₂O emissions compared to monoculture cropland systems (Díaz-Pinés et al., 2017; Horemans et al., 2019).

In the present study, we investigated the effects of converting cropland monoculture to

alley cropping agroforestry systems on soil N₂O fluxes at three sites in Germany, where the agroforestry systems combined crop rows and hybrid poplar SRC, and the poplar trees were unfertilized (Schmidt et al., 2021; Luo et al., 2022). We compared soil N₂O fluxes between cropland agroforestry and monoculture systems systematically over two years following different crop rotations and fertilization rates at each site. Our objectives were (1) to quantify the spatial-temporal dynamics of soil N₂O fluxes, and (2) to determine their controlling factors in cropland agroforestry and monoculture systems. We hypothesized that soil N₂O emission from unfertilized tree row will be lower than that from crop row. Therefore, when integrate agroforestry as a whole, soil N₂O emissions from agroforestry will be lower than from cropland monoculture systems.

2.2. Materials and methods

2.2.1. Experimental design and management practices

This study was carried out at three sites in Germany (Figure 2.1a) on loam Phaeozem (Dornburg, Thuringia), clay Cambisol (Wendhausen, Lower Saxony) and sandy Arenosol soils (Vechta, Lower Saxony) (Table S1). The average annual precipitation (2010–2019) at the three sites ranged 567–635 mm, and the average annual air temperature ranged 10–11 °C (Table S2.1). Each site had adjacent cropland agroforestry and monoculture systems. The agroforestry was established in 2007 (at the site on loam Phaeozem soil) and in 2008 (at the site on clay Cambisol soil) on the former monoculture systems by planting 12-m wide rows of fast-growing poplar (clone Max1, *Populus nigra* \times *P. maximowiczii*), used as feedstock for bioenergy production, alternated with 48-m wide crop rows (Figure 2.1b). The first harvest of aboveground biomass of these agroforestry trees was in January 2015 (at the site on loam Phaeozem soil) and in January 2014 (at the site on clay Cambisol soil). In addition, from an earlier study at our study sites on crops' nutrient response efficiency (NRE, measured in 2016 and 2017), both monoculture and agroforestry crop rows were at the nutrient saturation range in terms of fertilization rate and soil available nutrients (Schmidt et al., 2021). Thus, a followon experiment was established in March 2019 at the site with loam Phaeozem soil, whereby additional agroforestry and monoculture plots were included that did not have fertilization. At

this site, the crop in 2019 was summer barley (which had the lowest fertilization rates relative to other crops; Table 2.1), and the normally fertilized agroforestry crop rows and monoculture (Table 2.1) were contrasted with these unfertilized agroforestry crop rows and monoculture, all with the same experimental design as well as the rest of the management practices. At the site on sandy Arenosol soil, agroforestry was established in April 2019 by planting a 12-m wide poplar in the middle of the field and both sides had 48-m wide crop rows (Figure 2.1c). At each site, we established four replicate plots in both agroforestry and monoculture. In agroforestry, each replicate plot had four sampling locations: in the middle of the tree row, in the crop row at distances of 1 m, 7 m and 24 m from the edge of the tree row (Figure 2.1b, c). In monoculture, sampling points were located in the center of each replicate plot (Figure 2.1d).

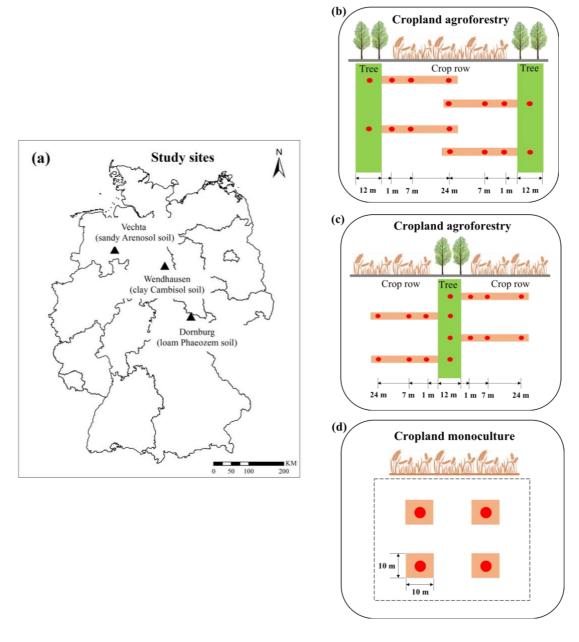


Figure 2.1 (a) Locations of the three study sites in Germany; (b) measurement layout (red points) in cropland agroforestry (at each replicate plot, sampling was conducted in the tree row and at 1 m, 7 m and 24 m within the crop row) at the sites on loam Phaeozem soil (Dornburg) and clay Cambisol soil (Wendhausen); (c) measurement layout in cropland agroforestry at the site on sandy Arenosol soil (Vechta); (d) measurement layout of four replicate plots in cropland monocultures at each of the three sites.

Table 2.1 Management practices in cropland agroforestry and monoculture systems at three sites in Germany during the cropping periods in2018–2019 and 2019–2020.

Soil type/site	Study period	Crop rotation	Sowing	Harvest	Fertilization date	Fertilization rate
						(kg N-P-K ha ⁻¹ yr ⁻¹)
loam Phaeozem	/ 2018–2019	Winter wheat	Oct 2017	Jul 2018	04.04.2018	133-0-0
Dornburg					17.05.2018	80-0-0
	2019-2020	Summer barley	Mar 2019	Jul 2019	01.04.2019	36-22-31
clay Cambisol	/ 2018–2019	Winter wheat	Oct 2017	Jul 2018	06.03.2018	70-0-0
Wendhausen					20.04.2018	60-0-0
					14.05.2018	36-0-0
	2019-2020	Corn	Apr 2019	Oct 2019	07.05.2019	101-0-0
sandy Arenosol	/ 2018–2019	Rye	Oct 2017	Jul 2018	08.02.2018	0-0-66
Vechta					10.02.2018	80-26-41
					22.03.2018	72-0-0
					09.05.2018	36-0-0
	2019-2020	Corn	Apr 2019	Sep 2019	24.04.2019	120-40-62
					03.05.2019	33-15-0

At each site, the agroforestry tree row did not receive fertilizer (which is the common practice of our farmer collaborators; Schmidt et al., 2021) whereas the agroforestry crop row had the same crops and agronomic practices as the monoculture (Table 2.1). Except for the follow-on comparisons from the same set-up but without fertilization at the site on loam Phaeozem soil, all crops and management practices (e.g., fertilization rates, fertilizer sources, sowing and harvesting; Table 2.1) in these field-based investigations were typical practices of our farmer collaborators. In the field, we observed that the fertilizer broadcaster drove at 12 m from the edge of the tree row; the fertilizers were applied for the entire 12 m length at each side of the broadcaster, and the broadcaster turned around to fertilize the remaining 24 m crop row. In the middle (24 m) of the agroforestry crop row, the fertilizers were applied with about 1 m overlapped, such that at 24 m the amount of fertilizers were likely more than the rest of the length of the crop row.

2.2.2. Soil N₂O flux measurement

Soil N₂O fluxes were measured monthly using vented static chambers (e.g., Corre et al., 2014; Hassler et al., 2015; Matson et al., 2017) at the three sites from March 2018 to January 2020 and from March 2019 to January 2020 for the follow-on comparison from the same set-up but without fertilization at the site on loam Phaeozem soil. At the site on sandy Arenosol soil, for the first measurement year (March 2018 to February 2019) prior to the establishment of agroforestry in April 2019, we measured N₂O fluxes in the pre-established eight sampling plots under monoculture. Due to logistical reasons, we were unable to carry out measurements in June or July 2018 (extreme dry period) and December 2018 or January 2019 (frozen soil). At each sampling point in each replicate plot, a chamber base made of polyvinyl chloride (0.04 m² in area) was inserted approximately 0.03 m into the soil. During the cropping season, these chamber bases were placed between the seeded rows. In the agroforestry crop row and monoculture, these chamber bases were installed on each measurement day and were removed after sampling in order to not hamper farmers' field activities. In the agroforestry tree row, the chamber bases were installed permanently as the tree rows were neither cultivated nor fertilized. On each measurement period, the chamber bases were covered for 32 minutes with

polyethylene hoods (total chamber volume was measured in all sampling days and was on average 10.5 L) that had a Luer-lock port for headspace gas sampling. In each chamber, four gas samples (25 mL each) were taken using a syringe at 2, 12, 22, and 32 minutes after closure and immediately injected into pre-evacuated glass vials with rubber septa (Exetainers, 12 mL; Labco Limited, Lampeter, UK). Each gas sample was analysed serially for N₂O (with an electron capture detector) and for CO₂ (with a methanizer and a flame ionization detector), using a gas chromatograph (SRI 8610C, SRI Instruments Europe GmbH, Bad Honnef, Germany).

We regarded the linear increase of CO₂ concentrations with chamber closure time as our reference for quality check of N₂O concentrations. All chamber measurements showed significant linear increases in CO₂ concentrations during the 32–minute chamber closure ($R^2 > 0.9$), justifying that all measured N₂O concentrations were valid from all chamber measurements. Soil N₂O fluxes were calculated from the linear change in concentrations over time, adjusted with measured air temperature and atmospheric pressure during the time of sampling. Annual N₂O emissions from each sampling location at each replicate plot were estimated using the trapezoidal interpolation between monthly measured fluxes and time intervals during March 2018–February 2019 and March 2019–January 2020 (the latter was ratioed to 365 days) (e.g. Corre et al., 2014; Hassler et al., 2017; Koehler et al., 2009).

To calculate the overall soil N₂O fluxes from the agroforestry as a whole, we used the weighting factors of the areal coverages of the tree row and crop row's sampling locations. The weighting factors were calculated by considering half of the widths of the tree row (6 m) and the crop row (24 m), totalling to 30 m, as the alternating tree and crop rows indicated that half of their widths represented each side of the rows (Figure 2.1b, c). The weighting factors were: 0.2 for the tree row (6 m/30 m), 0.13 for 1 m (4 m/30 m), 0.6 for 7 m (18 m/30 m) and 0.07 for 24 m (for 2 m/30 m; see section 2.1).

2.2.3. Soil controlling factors

Following each chamber measurement, soil temperature, WFPS and mineral N were determined. Soil temperature was measured in the top 5 cm using a GMH 1170 digital

thermometer (Greisinger electronic GmbH, Regen-stauf, Germany). At each sampling point, four intact soil cores (250 cm³ in volume) were taken in the top 5 cm. One soil core was measured for gravimetric moisture content by oven-drying at 105 °C for one day and used for determination of soil bulk density; the gravimetric moisture content was converted to WFPS, using the average of the repeatedly measured soil bulk density and a particle density of 2.65 g cm⁻³ for mineral soils. The remaining three soil cores were pooled and mixed thoroughly in the field, and a fresh soil sample (approx. 50 g) was put into prepared bottles containing 150 mL 0.5 M K₂SO₄ for mineral N extraction. Upon arrival at our laboratory, the extraction bottles were shaken for 1 h, filtered through 0.5 M K₂SO₄ pre-washed filter papers and extracts were immediately frozen until analysis. Extractable NH₄⁺ and NO₃⁻ were analyzed using continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany), where NH₄⁺ was determined by salicylate and dicloroisocyanuric acid reaction method (Autoanalyzer Method G-102-93) and NO₃⁻ by cadmium reduction method with NH₄Cl buffer (Auto-analyzer Method G-254-02) (Wen et al., 2017).

The general soil physical and chemical characteristics (texture, pH, organic carbon, total N and effective cation exchange capacity) were determined using standard methods as described in our previous work (Schmidt et al., 2021; Table S2.2).

2.2.4. Statistical analysis

Data of soil N₂O fluxes and soil factors (temperature, WFPS, NH₄⁺ and NO₃⁻) were tested for normality using Shapiro-Wilk test and homogeneity of variance using Levene's test. Parameters with non-normal distribution were log or square root transformed (i.e., N₂O, WFPS, NH₄⁺ and NO₃⁻). Differences in soil N₂O fluxes and soil factors between management systems (i.e., agroforestry sampling locations and monoculture) within each site and among sites for monoculture were tested using linear mixed-effects (LME) models (Crawley, 2007), with management system or site as fixed effect and sampling days and replicate plots as random effects. In the LME models, we included 1) a first-order temporal autoregressive process that assumes a decreasing correlation between measurements with increasing time distance (Zuur et al., 2009), and 2) a variance function that allows different variances of the fixed effect (Crawley, 2012). The best LME model was chosen based on the Akaike information criterion, supported with visual inspection of residuals of the model fit. To evaluate the differences in s soil N₂O fluxes between the whole agroforestry and the monoculture, the agroforestry was weighted by the areal coverage of the tree row and crop row sampling locations and LME tests were conducted as above. Significant differences were evaluated using the analysis of variance (ANOVA) with Fisher's least significant difference test for multiple comparisons at $p \le 0.05$. For soil physical and chemical characteristics measured once, differences between management systems were tested using a one-way ANOVA followed by Tukey HSD test or Kruskal-Wallis test (for variables with non-normal distributions). Spearman's rank correlation test (non-normal distribution of parameters) was conducted to assess the relationships between soil N₂O fluxes and the concurrently measured soil controlling factors (temperature, WFPS, mineral N), using the mean values of four replicate plots on each sampling day and analyzed over the entire study period. These correlation tests were conducted separately for the monoculture and agroforestry tree and crop rows in order to unravel which soil factors dominate at each management system. As the influence of WFPS on soil N2O fluxes could change during the cropping period following fertilization and after harvest, we further conducted Spearman's rank correlation test separately for the cropping season and after crop harvest. Correlation coefficients were considered significant at $p \le 0.05$. All statistical analyses were conducted using the open-source software R version 3.6.2 (R Core Team, 2019).

2.3. Results

2.3.1. Soil N₂O fluxes

Soil N₂O emissions peaked at agroforestry crop row and monoculture (Figure 2.2) following fertilizer applications (within March to May depending on the crops; Table 2.1) when the soil temperature was increasing (Figure S2.1a–c), the WFPS was between 30–55% (Figure S2.1d–f) and the mineral N levels were high (Figure S2.1g–l). Soil N₂O fluxes generally decreased after harvest towards fall and winter (Figure 2.2) as the soil temperature and mineral N decreased (Figure S2.1). A few exceptions were small pulses of soil N₂O emissions after wheat harvest at the sites on loam Phaeozem and clay Cambisol soils in July and August 2018 when NO₃⁻ and soil temperature were still high (Figure 2.2; Figure S2.1a, b, j, k). Also, in the clay Cambisol and sandy Arenosol soils following corn harvest in fall 2019, pulses of soil N₂O emissions occurred when WFPS remained high (Figure 2.2; Figure S2.1e, f). Farmers' practices on fertilization rates were largest for corn and lowest for barley (Table 2.1), and pulses of soil N₂O emissions following fertilization to corn were also larger than when the crop was barley (Figure 2.2).

At the loam Phaeozem soil, soil N₂O emissions during the two-year measurement period were lowest in the tree row and increased with increasing distances within the crop row with the highest emission at the 24 m (p < 0.04; Table 2.2); soil N₂O emissions from the monoculture were comparable to those at 1 m and 7 m in the agroforestry crop row (p > 0.16; Table 2.2). Similar pattern was observed in the follow-on experiment without fertilization in 2019–2020. In this follow-on experiment, soil N₂O emissions from the unfertilized agroforestry crop row were lower than the fertilized agroforestry crop row (p < 0.02; Table 2.2), whereas emissions were comparable between the unfertilized and fertilized monocultures (p = 0.72; Table 2.2). At the clay Cambisol soil, similar spatial pattern was observed from the agroforestry tree row and the increasing distances within the crop row during the two-year measurement period (p < 0.01; Table 2.2); soil N₂O emissions from the monoculture were generally comparable to those from the 3 m and 24 m in the agroforestry crop row (p > 0.11; Table 2.2). At the sandy Arenosol soil, where the trees were just established in April 2019 (Table S2.1) and the crop was corn

with typically high fertilization rate (Table 2.1), the resulting large soil N₂O emissions did not differ between the monoculture and the first-year agroforestry (p = 0.07; Table 2.2).

Comparing between the entire agroforestry (weighted by the areal coverages of the tree and crop rows) and monoculture, soil N₂O emissions from agroforestry were comparable to those from monoculture in the loam Phaeozem soil (p = 0.06 and 0.69; Table 2.2), wherein wheat during the first year had the typical split fertilizer applications and the barley during the second year had commonly low fertilization rate (Table 2.1). In the clay Cambisol soil, the entire agroforestry had comparable soil N₂O emissions with the monoculture only during the first year with wheat that had split fertilizations (p = 0.16; Table 2.2) while during the second year with corn that had one-time large fertilization (Table 2.1), soil N₂O emissions were lower in agroforestry than in monoculture (p = 0.04; Table 2.2). Across sites, soil N₂O emissions did not differ among the cropland monocultures in 2018–2019 (p = 0.75; Table 2.2) with wheat and rye that typically had split fertilizer applications; in 2019–2020, soil N₂O emissions were larger on the clay Cambisol and sandy Arenosol soils (p < 0.01; Table 2.2) that had corn with one-time large fertilization than those on the loam Phaeozem soil that had barley with low fertilization rate (Table 2.1).

Across sites, soil annual N₂O emissions in agroforestry as a whole (weighted by the areal coverages of the tree and crop rows) ranged from 0.21 to 2.73 kg N ha⁻¹ yr⁻¹ and the monoculture croplands ranged from 0.34 to 3.00 kg N ha⁻¹ yr⁻¹ (Table 2.2). Soil annual N₂O emissions generally increased with increasing N fertilization rates, which were highest when the crop was corn with one-time fertilizer application even on the sandy soil (Figure S2.3) that showed low WPFS (Table 2.3; Figure S2.1f). The lowest annual N₂O emission was observed when the crop was barley (Figure S2.3), which typically had the lowest fertilization rate (Table 2.1). On the other hand, although wheat and rye had in total highest fertilization rates, their applications were split into two to three during spring, resulting to lower soil N₂O emissions than when the crop was corn (Figure S2.3). The ratios of annual N₂O emissions to annual N fertilization rates were 0.3% for rye, 0.4% for wheat, 1.2% for barley and 2.1% for corn.

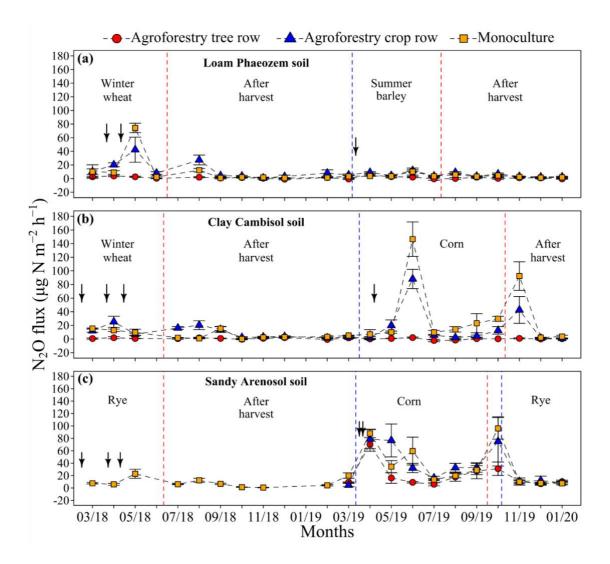


Figure 2.2 Monthly mean (\pm SE, n = 4) soil N₂O fluxes in cropland agroforestry tree row, crop row, and monoculture at three sites in Germany. Soil N₂O fluxes in agroforestry crop row were area-weighted average of the 1 m, 7 m and 24 m sampling locations. Black arrows indicate fertilizer application in the agroforestry crop row and monoculture only (rates are given in Table 1); tree rows were commonly unfertilized. Blue vertical lines indicate sowing; red vertical lines indicate harvest.

Soil type /	Management N ₂ O fluxes		Annual N2O er	nission	
Site	system	$(\mu g \ N \ m^{-2} \ h^{-1})$		(kg N ha ⁻¹ yr ⁻¹)
		2018-2019	2019-2020	2018-2019	2019-2020
loam Phaeozem /	Tree row	1.5 ± 0.6^{d}	$1.2 \pm 1.0^{\circ}$	0.11 ± 0.02	0.11 ± 0.03
Dornburg	1 m crop row	7.6 ± 4.0^{cd}	3.3 ± 1.0^{b}	0.59 ± 0.33	0.29 ± 0.06
(normal fertilization)	7 m crop row	11.4 ± 4.1^{b}	5.5 ± 1.1^{b}	0.94 ± 0.33	0.48 ± 0.03
	24 m crop row	$36.1\pm17.6^{\rm a}$	11.4 ± 4.3^{a}	3.73 ± 0.69	0.98 ± 0.03
	Whole agroforestry	$10.6\pm3.5^{\rm A}$	$4.7\pm0.9^{\rm A}$	0.91 ± 0.27	0.41 ± 0.02
	Monoculture	12.0 ± 7.9^{bcA}	$3.9 \pm 1.1^{\text{bA}}$	0.89 ± 0.09	0.34 ± 0.02
loam Phaeozem /	Tree row		$1.1 \pm 1.0^{\circ}$		0.04 ± 0.03
Dornburg	1 m crop row		1.6 ± 0.8^{bc}		0.13 ± 0.03
(without fertilization)	7 m crop row		2.8 ± 1.2^{bc}		0.23 ± 0.06
	24 m crop row		$7.7\pm3.7^{\rm a}$		0.66 ± 0.13
	Whole agroforestry		$2.7\pm0.9^{\rm A}$		0.21 ± 0.05
	Monoculture		5.0 ± 2.9^{abA}		0.38 ± 0.09
clay Cambisol /	Tree row	0.9 ± 2.9^{b}	$0.3 \pm 0.3^{\rm c}$	0.07 ± 0.04	0.02 ± 0.04

Table 2.2 Mean and annual (\pm SE, n = 4) soil N₂O emissions from cropland agroforestry and monoculture systems at three sites in Germany, measured in 2018–2019 (from March 2018 to February 2019) and 2019–2020 (from March 2019 to January 2020).

Wendhausen	1 m crop row	12.0 ± 4.8^{a}	8.5 ± 4.7^{b}	0.96 ± 0.43	0.84 ± 0.17
	7 m crop row	9.9 ± 3.0^{a}	18.2 ± 10.5^{ab}	0.76 ± 0.17	1.79 ± 0.23
	24 m crop row	$12.7\pm4.8^{\rm a}$	16.9 ± 8.5^{ab}	0.96 ± 0.17	1.64 ± 0.25
	Whole agroforestry	$8.6\pm2.4^{\rm A}$	13.2 ± 7.5^B	0.66 ± 0.15	1.30 ± 0.14
	Monoculture	6.5 ± 2.2^{aA}	29.6 ± 14.8^{aA}	0.49 ± 0.07	2.92 ± 0.45
sandy Arenosol /	Tree row		19.5 ± 6.4^{a}		1.77 ± 0.27
Vechta	1 m crop row		48.8 ± 19.8^{a}		3.90 ± 0.77
	7 m crop row		31.5 ± 8.8^{a}		2.76 ± 0.58
	24 m crop row		$34.4 \pm 11.3^{\text{a}}$		2.96 ± 0.29
	Whole agroforestry		$32.1\pm8.5^{\rm A}$		2.73 ± 0.44
	Monoculture	$6.3\pm1.9^{\dagger}$	35.0 ± 10.6^{aA}	$0.51^\dagger\pm0.09$	3.00 ± 0.31

Note: For each site, means with different lowercase letters indicate significant differences between the monoculture and sampling locations within the agroforestry system and different capital letters indicate significant differences between the whole agroforestry (weighted by the areal coverage of the tree row and crop row sampling locations; see section 2.2) and monoculture (Linear mixed-effects model with Fisher's LSD test at $p \le 0.05$). Annual soil N₂O emissions are calculated using the trapezoidal interpolation between fluxes and time intervals during the measurement periods of 2018–2019 and 2019–2020, and hence were not tested statistically.

[†]Measurements on sandy Arenosol soil in 2018–2019 were conducted prior to agroforestry establishment in April 2019 and all replicate plots were still under monoculture, n = 8.

Table 2.3 Mean (\pm SE, n = 4) soil temperature, WFPS, NH₄⁺ and NO₃⁻ concentrations in the top 5-cm depth in cropland agroforestry and monoculture systems at three agroforestry sites in Germany, measured in 2018–2019 (from March 2018 to February 2019) and 2019–2020 (from March 2019 to January 2020)

Soil type/site	Management	Soil temperature (°C)		WFPS (%)		NH4 ⁺ (mg N kg ⁻¹)		NO3 ⁻ (mg N kg ⁻¹)	
	system	2018-2019	2019–2020	2018-2019	2019-2020	2018-2019	2019-2020	2018-2019	2019-2020
loam Phaeozem /	Agroforestry								
Dornburg	Tree row	10 ± 2^{a}	10 ± 2^{a}	47 ± 4^{a}	51 ± 5^{ab}	2 ± 1^{b}	3 ± 1^{a}	1 ± 0^{d}	1 ± 0^{b}
(normal	1 m crop row	10 ± 2^{a}	11 ± 2^{a}	41 ± 4^{ab}	50 ± 4^{ab}	6 ± 3^{b}	2 ± 1^{b}	7 ± 2^{c}	4 ± 2^{a}
fertilization)	7 m crop row	11 ± 2^{a}	12 ± 2^{a}	39 ± 4^{bc}	$58\pm 6^{\mathrm{a}}$	22 ± 11^{ab}	2 ± 1^{b}	11 ± 4^{bc}	3 ± 1^{a}
	24 m crop row	11 ± 2^{a}	12 ± 2^{a}	40 ± 3^{b}	54 ± 5^{ab}	57 ± 28^{a}	3 ± 2^{ab}	28 ± 9^{a}	5 ± 2^{a}
	Monoculture	10 ± 2^{a}	12 ± 2^{a}	33 ± 2^{c}	46 ± 4^{b}	30 ± 16^{ab}	2 ± 1^{b}	24 ± 9^{ab}	6 ± 2^{a}
loam Phaeozem /	Agroforestry								
Dornburg	Tree row		9 ± 2^{a}		45 ± 4^{b}		3 ± 1^{a}		1 ± 0^{b}
(without	1 m crop row		10 ± 2^{a}		44 ± 4^{b}		$1\pm1^{\mathrm{b}}$		2 ± 1^{a}
fertilization)	7 m crop row		10 ± 2^{a}		54 ± 4^{a}		$1\pm1^{\mathrm{b}}$		2 ± 1^{a}
	24 m crop row		10 ± 2^{a}		$54\pm5^{\rm a}$		$1\pm1^{\mathrm{b}}$		3 ± 1^{a}
	Monoculture		12 ± 2^{a}		44 ± 5^{b}		$1\pm1^{\mathrm{b}}$		4 ± 1^{a}
clay Cambisol /	Agroforestry								

Wendhausen	Tree row	11 ± 2^{a}	11 ± 2^{a}	48 ± 4^{a}	50 ± 4^{a}	3 ± 1^{a}	2 ± 0^{a}	2 ± 1^{b}	$1 \pm 0^{\rm c}$
	1 m crop row	11 ± 2^{a}	12 ± 2^{a}	42 ± 3^{ab}	43 ± 3^{b}	4 ± 2^{a}	5 ± 3^{a}	23 ± 8^{a}	18 ± 7^{b}
	7 m crop row	11 ± 2^{a}	12 ± 2^{a}	42 ± 4^{ab}	45 ± 3^{ab}	8 ± 5^{a}	9 ± 5^{a}	14 ± 3^{a}	41 ± 15^{ab}
	24 m crop row	11 ± 2^{a}	12 ± 2^{a}	40 ± 3^{bc}	46 ± 4^{ab}	8 ± 5^{a}	8 ± 6^{a}	18 ± 4^{a}	31 ± 12^{ab}
	Monoculture	11 ± 2^{a}	13 ± 2^{a}	35 ± 4^{c}	40 ± 3^{b}	8 ± 4^{a}	10 ± 6^{a}	16 ± 3^{a}	39 ± 14^{a}
sandy Arenosol /	Agroforestry								
Vechta	Tree row		13 ± 2^{a}		31 ± 3^{a}		1 ± 1^{a}		20 ± 9^{a}
	1 m crop row		13 ± 2^{a}		31 ± 3^{a}		$9\pm 6^{\mathrm{a}}$		43 ± 20^{a}
	7 m crop row		13 ± 2^{a}		31 ± 4^{a}		10 ± 7^{a}		55 ± 23^a
	24 m crop row		13 ± 2^{a}		30 ± 3^{a}		8 ± 5^{a}		57 ± 25^{a}
	Monoculture	$11\pm2^\dagger$	12 ± 2^{a}	$32\pm3^{\dagger}$	32 ± 4^{a}	$10\pm7^\dagger$	4 ± 2^{a}	$9\pm5^{\dagger}$	35 ± 15^{a}

Note: For each site, means followed by different lowercase letters indicate significant differences between the monoculture and sampling locations within the agroforestry system (linear mixed-effects model with Fisher's LSD test at $p \le 0.05$).

[†] Measurements on sandy Arenosol soil in 2018–2019 were conducted prior to agroforestry establishment in April 2019 and all replicate plots were still under monoculture, n = 8.

2.3.2. Soil controlling factors

Soil temperature showed similar seasonal patterns within each site, which generally increased towards spring and summer and decreased in fall and winter, ranging from 0 to 24 °C throughout the two measurement years (Figure S2.1a–c; Figure S2.2b). WFPS showed the opposite trends as temperature, with the highest WFPS observed in winter (approx. 50-90%; Figure S2.1d–f) and the lowest in summer (approx. 10-30%; Figure S2.1d–f). Across the measurement period, WFPS ranged from 20 to 90%, 15 to 65% and 10 to 55% in the loam Phaeozem, clay Cambisol and sandy Arenosol soils, respectively (Figure S2.1d–f; Figure S2.2c). Soil mineral N (NH₄⁺ and NO₃⁻) concentrations generally increased following fertilization (Figure S2.1g–l), and after two months remained at low levels (0.09 mg N kg⁻¹).

Soil temperature did not differ between management systems within each site (p > 0.94; Table 2.3). At the loam Phaeozem soil, WFPS was highest in the tree row, followed by the crop row and lowest in the monoculture in 2018–2019 (p < 0.01; Table 2.3), whereas in 2019–2020, WFPS in the monoculture was comparable with the tree row and lower than in the crop row (p = 0.01; Table 2.3); soil mineral N was generally lower in the tree row than in the crop row and monoculture (p < 0.01; Table 2.3), except for the converse pattern of soil NH₄⁺ in 2019–2020 (Table 2.3). At the clay Cambisol soil, WFPS decreased in the order of tree row, crop row and monoculture during the measurement period (p < 0.01; Table 2.3); soil NH₄⁺ did not differ between management systems (p > 0.35; Table 2.3) whereas soil NO₃⁻ in the tree row was lower compared to the crop row and monoculture (p < 0.01; Table 2.3). At the sandy Arenosol soil, soil NH₄⁺ and NO₃⁻ concentrations and WFPS did not differ between management systems (p > 0.11; Table 2.3).

Table 2.4 Spearman rank correlations of soil N₂O fluxes (μ g N m⁻² h⁻¹) with soil temperature (°C), water-filled pore space (WFPS), and total (NH₄⁺ + NO₃⁻) mineral N (mg N kg⁻¹), measured in the top 5 cm depth, across monthly measurements from March 2018 to January 2020.

Soil type / Site	Managamanta	Tomporatura	WFPS	Mineral N
Soil type / Site	Managements	Temperature	WFF5	winner at N
loam Phaeozem /	Whole agroforestry $(n = 84)$	0.41**	-0.09	0.58**
Dornburg	Tree row $(n = 21)$	0.35	0.16	0.67**
(normal fertilization)	Crop row $(n = 63)$	0.49**	-0.08	0.53**
	Monoculture ($n = 21$)	0.71**	0.18	0.46*
loam Phaeozem /	Whole agroforestry $(n = 44)$	0.27	-0.03	0.31*
Dornburg	Tree row $(n = 11)$	-0.12	-0.13	0.45
(without fertilization)	Crop row $(n = 33)$	0.35*	-0.08	0.30
	Monoculture ($n = 11$)	0.44	-0.31	0.55
clay Cambisol /	Whole agroforestry $(n = 84)$	0.28**	-0.18	0.52**
Wendhausen	Tree row $(n = 21)$	-0.11	0.26	0.25
	Crop row $(n = 63)$	0.38**	-0.10	0.39**
	Monoculture ($n = 21$)	0.19	0.01	0.45*
sandy Arenosol /	Whole agroforestry $(n = 44)$	0.38*	-0.13	0.46**
Vechta	Tree row $(n = 11)$	-0.01	0.36	-0.14
	Crop row $(n = 33)$	0.48**	-0.25	0.52**
	Monoculture ($n = 20$)	0.47*	-0.07	0.39*

Note: The correlation tests were conducted using the means of the four replicates on each sampling day, and thus n is the measurement days during the two-year study period.

* $p \le 0.05$, ** $p \le 0.01$.

2.3.3. Relationships between soil N₂O fluxes and controlling factors

Across the measurement period, soil N₂O emissions were largely correlated with mineral N in the fertilized agroforestry crop rows and monocultures (Table 2.4). Soil N₂O emissions was not correlated with mineral N only in the tree rows where no fertilizer was applied. Thus, for agroforestry as a whole (weighted by the areal coverages of the tree and crop rows), the correlation between N₂O and mineral N was brought about by the range of conditions from unfertilized tree rows and the fertilized crop rows (Table 2.4). Similarly, there was no relationship detected between soil N₂O emission and mineral N in the unfertilized agroforestry crop row and monoculture on the loam Phaeozem soil (Table 2.4). Although we detected a positive correlation between soil temperature and N₂O emission (Table 2.4), this was not only by soil temperature but also confounded by its auto-correlation with total mineral N (Table S2.3). At each site, there was no clear relationship detected between soil N₂O emission and WFPS (Table 2.4; Table S2.3). However, when separating the influence of WFPS during the fertilization effect in the cropping season on soil N2O emission, we detected positive relationships between soil N₂O emission and WFPS during the cropping seasons on the loam Phaeozem and clay Cambisol soils (Figure S2.4), and no correlations after harvest at all sites (Figure S2.4).

2.4. Discussion

2.4.1. Soil N₂O emissions from cropland agroforestry and monoculture systems

Monthly measurements of soil N₂O fluxes in European and North American cropland agroforestry systems over two years have never been documented in literature. Soils in cropland agroforestry and monoculture systems were N₂O sources at our sites. Annual and monthly soil N₂O emissions from the entire agroforestry (weighted by the areal coverages of the tree and crop rows) and monoculture systems (Table 2.2) were within the range (0.19–4.70 kg N ha⁻¹ yr⁻¹) reported for cropping systems in Germany (Table S2.4). Although Franzluebbers et al. (2017) measured soil N₂O emission from agroforestry tree and crop row for a whole year in eastern USA, with average annual emissions of 0.2 ± 0.1 and 1.2 ± 0.4 kg

N ha⁻¹ yr⁻¹ from agroforestry tree row and crop row, they did not compare the difference between agroforestry as a whole and monoculture systematically. Our systematic comparison revealed that soil N₂O emissions were generally lower from the entire agroforestry than from monoculture system. This was caused by the low soil N₂O emissions from the agroforestry tree rows that were within the range ($\sim 2.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) reported for hybrid poplar SRC soils in Germany (Díaz-Pinés et al., 2017; Kern et al., 2010; Walter et al., 2015) and other European temperate regions (0.24 kg N ha⁻¹ yr⁻¹, Harris et al., 2017; 0.23–7.38 kg N ha⁻¹ yr⁻¹, Horemans et al., 2019). Similarly, Beaudette et al. (2010) and Wolz et al. (2018b) reported that potential nitrification rates and soil N₂O emissions were decreased in tree-based intercropping systems of temperate North America regions during the crop growing season. Furthermore, our results confirm the assumption that whole-year soil N₂O measurements are important for a systematic comparison of soil N₂O emissions between entire agroforestry and monoculture systems because soil N₂O emissions during non-growing seasons highly contribute to annual soil N₂O emissions (Shang et al., 2020).

2.4.2. Environmental and management effects on soil N₂O emissions

Soil N₂O emissions from agroforestry crop rows and monocultures were driven by a combination of environmental (soil temperature, moisture and mineral N) and management (timing and magnitude of fertilizer application, crop type, rotation and harvest) effects. According to the hole-in-the-pipe (HIP) model (Davidson et al., 2000), soil N₂O production is primarily controlled by soil N availability, as indicated by positive correlations with soil mineral N concentrations across all sites and management systems (Table 2.4; Table S2.3). Consequently, soil N availability and soil N₂O emissions increased substantially in the crop rows following fertilizer application in spring and summer (Figure 2.2; Figure S2.1). Most of the produced soil N₂O may be derived from nitrification since WFPS were generally below 60% that favours nitrifier activity (Corre et al., 2014; Fan and Yoh, 2020). Soil aeration status, as indicated by WFPS, became the second level of control (Davidson et al., 2000) on the loam Phaeozem and clay Cambisol soils mainly during cropping seasons when soil temperatures and levels of mineral N were high, as indicated by positive relationships between soil N₂O emissions and WFPS (Figure S2.4). Low temperatures in fall and winter reduced microbial

activity and soil N cycling processes (Butterbach-Bahl et al., 2013), hence mineral N and soil N₂O emissions decreased (Figure 2.2; Figure S2.1). Despite low temperatures, low C/N corn residues and high values of WFPS were sufficient to increase soil N availability (i.e. mineral N), stimulate N₂O production and to create pronounced N₂O emission peaks in the monoculture and cropland agroforestry soils in the clay Cambisol and sandy Arenosols sites after harvests in fall 2019 (Figure 2.2). Similar results were also observed in other field studies, where left-over crop residue is a common practice to increase nutrient availability by local farmers (Akiyama et al., 2020; Pugesgaard et al., 2017).

Tree rows did not receive any fertilizer input at our study sites. Hence, we attribute the lower soil N₂O emissions from tree compared to crop rows and monocultures to the increased tree-microbial competition for soil available N (Abalos et al., 2016; Corre et al., 2014). This competition decreased with increasing distance from the tree row. Concurrently, soil available N increased further due to fertilizer input. However, the small trees at our youngest agroforestry site (sandy Arenosol soil) were incapable to sufficiently increase competition for available N and to reduce soil N₂O emissions, which confirms observations by Horemans et al., (2019) who suggested that tree rows in agroforestry systems may start to reduce soil N₂O emissions only few years after establishment.

Fertilization practices and crop type also influence soil N₂O emissions (Maul et al., 2019). Wrong fertilization practices caused 2–35% more soil annual N₂O emissions from agroforestry as a whole than from their adjacent monoculture in the loam Phaeozem and clay Cambisol sites in 2018–2019 (Table 2.2), because the fertilizer broadcaster had inadvertently overlapped the fertilization leading to high emission at the 24 m crop row. However, at the sites on clay Cambisol and sandy Arenosol soils, ratios of annual N₂O emissions to annual N fertilization rates were highest when crops were corn and lower when barley, wheat and rye that may be attributed to high soil N availability following high rates of one-time fertilizer application for corn and lower rates of one and two to three split fertilizer applications for wheat, rye and barley (Figure 2.1). Similarly, Laville et al. (2011) and Senbayram et al. (2014) reported higher soil N₂O emissions during corn than barley or wheat seasons. This difference may additionally originate from low N uptake of corn seedlings at the start of the growing season in spring and

early summer, when soil mineral N levels were highestand more available N may be used for N₂O production. In contrast, the crops winter wheat or rye were in their most productive growth stage in spring, which may simulate N uptake and reduce N₂O emissions. However, the low amount of N fertilizer was insufficient to stimulate soil N₂O emissions from monoculture at the site on loam Phaeozem soil in 2019–2020 (Table 2.1) because high "background" soil N₂O emissions from nitrification following mineralization of soil organic N may have masked any N-fertilizer effect (Barton et al., 2008).

2.4.3. Implications for cropland monoculture to cropland agroforestry conversion on soil N₂O fluxes

In summary, our systematic comparison of soil N₂O emissions from temperate cropland agroforestry and monoculture systems revealed low soil N₂O emissions from unfertilized tree rows to be responsible for decreased soil N₂O emissions following conversion of cropland monoculture to agroforestry, which supported our hypotheses. This reduction in soil N₂O emissions may only occur in mature agroforestry systems, where tree rows induce strong tree-microbial competition for nitrogen, such that microbial N₂O production is reduced. Furthermore, crop type and timing and magnitude of fertilizer application were responsible for substantial soil N₂O emissions from our cropland agroforestry area, our findings suggest that improved system management (e.g. optimal adjustments of the areal coverages between tree and crop rows) and optimized fertilizer input will enhance the potential of cropland agroforestry for mitigating N₂O emissions. Furthermore, our results emphasize the need to investigate soil N₂O fluxes following conversion of cropland monoculture to agroforestry for much prolonged periods of crop rotation and fertilizer application, to identify appropriate management practices for the reduction of soil N₂O emissions from agriculture.

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Appendix

Table S2.1 Site characteristics and management practices in cropland agroforestry and monoculture systems at three sites in Germany.

Study site	Dornburg	Wendhausen	Vechta
Location	51°00′40″ N, 11°38′46″ E	52°20′00″ N, 10°37′55″ E	52°45′29″ N, 8°32′5″ E
Elevation (m above sea level)	289	82	38
Soil type	loam Phaeozem	clay Cambisol	sandy Arenosol
Mean annual air temperature (2010–2019)	$10.7\pm0.3~^{\circ}C^{a}$	10.7 ± 0.3 °C ^b	$10.1 \pm 0.1 \ ^{\circ}C^{c}$
Mean annual precipitation (2010-2019)	$567 \pm 32 \text{ mm}^{a}$	$587 \pm 41 \ mm^b$	$635 \pm 35 \text{ mm}^{c}$
Year of establishment of agroforestry system	2007	2008	2019
First harvest of trees	January 2015	January 2014	none
Cropping history	2016: summer barley	2016: winter rapeseed	2016: corn
	2017: winter rapeseed	2017: winter wheat	2017: potato
	2018: winter wheat	2018: winter wheat	2018: rye
	2019: summer barley	2019: corn	2019: corn

^{a, b, c} Climate station at Jena (station ID: 2444), Braunschweig (station ID: 662), and Diepholz (station ID: 963) of the German Meteorological Service.

Table S2.2 Soil physical and biochemical characteristics (mean \pm SE, n = 4 plots for the loam Phaeozem and clay Cambisol soils, n = 8 plots for the sandy Arenosol soil) in the top 30 cm of cropland agroforestry tree row, crop row and monoculture at three sites in Germany.

Soil type/	Management	Soil tex	ture (%)		Bulk density	pH (1: 4 soil	Organic	Total N	C: N	Effective cation
site	system				-(g cm ⁻³)	-H2O ratio)	C (kg m ⁻²)	(kg m ⁻²)	ratio	exchange capacity
		Sand	Silt	Clay						(mmolc kg ⁻¹)
loam Phaeozem	/ Agroforestry									
Dornburg	Tree row	4 ± 0^{b}	76 ± 01^{a}	$20\pm1^{\text{b}}$	1.1 ± 0.0^{a}	$6.5\pm0.1^{\circ}$	$5.1\pm0.5^{\rm a}$	$0.5\pm0.0^{\mathrm{a}}$	10 ± 0^{a}	152 ± 5^{b}
	Crop row	4 ± 0^{b}	72 ± 01^{a}	$24\pm1^{\text{b}}$	1.1 ± 0.0^{a}	6.7 ± 0.0^{b}	$4.3\pm0.0^{\rm a}$	$0.5\pm0.0^{\mathrm{a}}$	9 ± 0^{ab}	159 ± 3^{b}
	Monoculture	11 ± 1^{a}	51 ± 3^{b}	38 ± 2^{a}	$1.0\pm0.0^{\rm b}$	7.9 ± 0.1^{a}	$3.9\pm0.7^{\rm a}$	0.5 ± 0.1^{a}	8 ± 0^{b}	590 ± 101^{a}
clay Cambisol /	Agroforestry									
Wendhausen	Tree row	18 ± 3^{a}	47 ± 4^{a}	35 ± 2^{a}	1.0 ± 0.0^{a}	7.1 ± 0.2^{a}	$7.0\pm0.3^{\text{a}}$	$0.7\pm0.0^{\mathrm{a}}$	11 ± 0^{a}	350 ± 75^{a}
	Crop row	18 ± 3^{a}	44 ± 3^{a}	38 ± 2^{a}	$1.0\pm0.0^{\mathrm{a}}$	7.3 ± 0.2^{a}	6.5 ± 0.2^{ab}	$0.7\pm0.0^{\mathrm{a}}$	10 ± 0^{b}	366 ± 100^{a}
	Monoculture	27 ± 2^{a}	29 ± 4^{b}	44 ± 3^{a}	$1.0\pm0.0^{\mathrm{a}}$	7.3 ± 0.1^{a}	5.8 ± 0.1^{b}	0.6 ± 0.0^{a}	10 ± 0^{b}	298 ± 10^{a}
sandy Arenosol	/ Monoculture	80 ± 1	13 ± 1	7 ± 1	1.3 ± 0.0	6.1 ± 0.4	6.9 ± 0.3	0.5 ± 0.0	13 ± 0	43 ± 4
Vechta										

Note: Soil characteristics were measured in 2016 for the loam Phaeozem soil, 2019 for the clay Cambisol soil, and 2018 for the sandy Arenosol soil prior to agroforestry establishment in April 2019. For each site, means followed by different lowercase letters indicate significant differences between management systems (ANOVA with Tukey HSD or Kruskal-Wallis test with multiple comparison extension at $p \le 0.05$).

Table S2.3 Spearman rank correlations between soil N₂O fluxes (μ g N m⁻² h⁻¹), soil temperature (°C), water-filled pore space (WFPS), and total (NH₄⁺ + NO₃⁻) mineral N (mg N kg⁻¹), measured in the top 5 cm depth, across management systems at each site from March 2018 to January 2020.

Soil type / Site		Soil temperature	WFPS	Mineral N
loam Phaeozem /	Soil N2O flux	0.47**	-0.04	0.54**
Dornburg ($n = 105$) (normal fertilization)	Soil temperature		-0.27**	0.34**
(WFPS			-0.24*
loam Phaeozem /	Soil N ₂ O flux	0.31*	-0.17	0.41**
Dornburg ($n = 55$) (without fertilization)	Soil temperature		-0.47**	0.52**
(WFPS			-0.45**
clay Cambisol /	Soil N2O flux	0.28**	-0.17	0.50**
Wendhausen ($n = 105$)	Soil temperature		-0.54**	0.60**
	WFPS			-0.34**
sandy Arenosol /	Soil N ₂ O flux	0.43**	-0.11	0.43**
Vechta ($n = 64$)	Soil temperature		-0.58**	0.79**
	WFPS			-0.61**

Note: The correlation tests were conducted using the means of the four replicates on each sampling day, and thus *n* is the measurement days during the two-year study period. * $p \le 0.05$, ** $p \le 0.01$.

Author	Soil type	Crop type	Period and frequency	Method	N applied	N ₂ O emission
			of measurement		(kg ha ⁻¹ yr ⁻¹)	(kg N ha ⁻¹ yr ⁻¹)
This study	Loam Phaeozem	winter wheat	March 2018 – February	Vented static	213	0.89
			2019 (monthly)	chamber		
This study	Loam Phaeozem	summer barley	March 2019 – January	Vented static	36	0.34
			2020 (monthly)	chamber		
This study	loam Phaeozem	summer barley	March 2019 – January	Vented static	0	0.38
			2020 (monthly)	chamber		
This study	Clay Cambisol	winter wheat	March 2018 – January	Vented static	166	0.49
			2020 (monthly)	chamber		
This study	Clay Cambisol	corn	March 2019 – January	Vented static	101	2.92
			2020 (monthly)	chamber		
This study	Sandy Arenosol	rye	March 2018 – January	Vented static	188	0.63
			2020 (monthly)	chamber		
This study	Sandy Arenosol	corn	March 2019 – January	Vented static	153	3.00
			2020 (monthly)	chamber		
Kesenheimer	Sandy loam Luvisol	winter oilseed rape	January 2013 –	Closed chamber	180	0.19 – 1.13
et al., 2021		– winter wheat	December 2015			
		– winter barley	(weekly)			
Kesenheimer	Silty loam Haplic	winter oilseed rape	January 2013 –	Closed chamber	180	0.54 - 1.40

Table S2.4 Annual soil N₂O emissions from croplands in Germany, measured in-situ with multiple measurement periods.

et al., 2021	Luvisol	– winter wheat	December 2015			
		– winter barley	(weekly)			
Kesenheimer	Sandy loam	winter oilseed rape	January 2013 –	Closed chamber	180	0.98 - 1.88
et al., 2021	Luvisol/Anthrosol	– winter wheat	December 2015			
		– winter barley	(weekly)			
Kesenheimer	Silty loam Haplic	winter oilseed rape	January 2013 –	Closed chamber	180	2.23 - 3.53
et al., 2021	Chernozem	– winter wheat	December 2015			
		– winter barley	(weekly)			
Helfrich et al.,	Sandy loam	maize	May 2010 – April 2012	Vented static	0	3.10 - 3.90
2020	Planosol		(weekly)	chamber		
Herr et al., 2019	Silt loam Luvisol	maize	May 2015 – May 2017	Vented static	0	2.80
			(weekly)	chamber		
Herr et al., 2019	Silt loam Luvisol	maize	May 2015 – May 2017	Vented static	180	4.70
			(weekly)	chamber		
Weller et al.,	Silt loam Luvisol-	maize	April 2012 – April	Automated static	0	0.79
2019	Anthrosol		2013 (continuously)	chamber		
Weller et al.,	Silt loam Luvisol-	maize	April 2012 – April	Automated static	177	1.27
2019	Anthrosol		2013 (continuously)	chamber		
Walter et al.,	Silt loam Cambisol	winter wheat	November 2012 –	Vented static	252	1.54
2015			November 2013	chamber		
			(bi-weekly)			

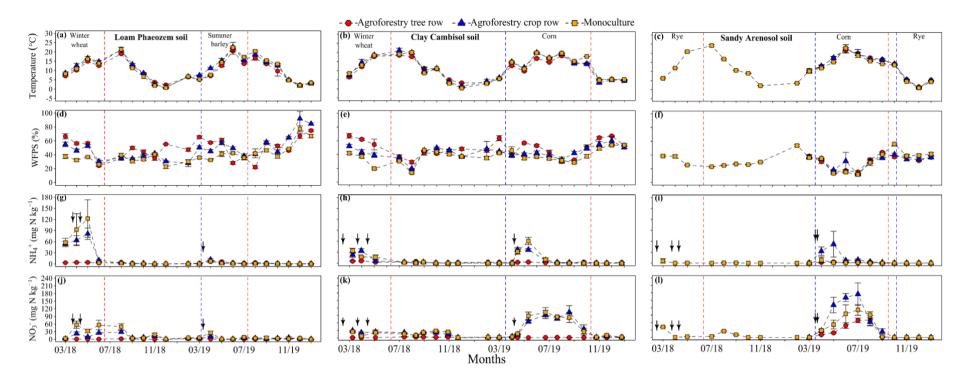


Figure S2.1 Monthly mean (\pm SE, *n* = 4) soil temperature (a, b, c), water-filled pore space (WFPS, d, e, f), NH₄⁺ (g, h, i) and NO₃⁻ (j, k, l) in the top 5 cm depth in agroforestry tree row, crop row and monoculture at three sites in Germany. Values of agroforestry crop row were area-weighted average of the 1 m, 7 m and 24 m sampling locations. Black arrows indicate fertilizer application in the agroforestry crop row and monoculture; tree rows were commonly unfertilized. Blue vertical lines indicate sowing; red vertical lines indicate harvest.

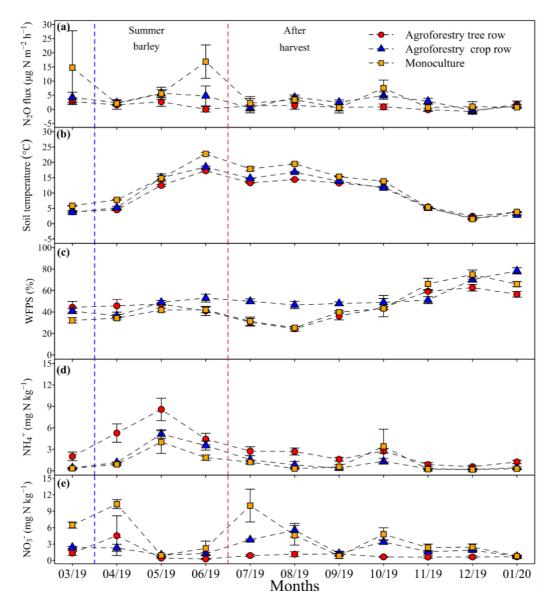


Figure S2.2 Monthly mean (\pm SE, n = 4) soil N₂O fluxes (a), temperature (b), water-filled pore space (WFPS, c), NH₄⁺ (d) and NO₃⁻ (e) in the top 5 cm depth in cropland agroforestry tree row, crop row and monoculture in loam Phaeozem soil (Dornburg site) without fertilizer application in 2019–2020. Blue vertical line indicates sowing; red vertical line indicates harvest.

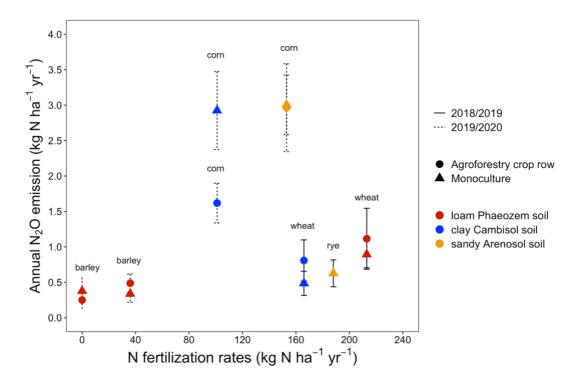


Figure S2.3 Relationships between annual soil N_2O emissions in agroforestry crop row and monoculture with N fertilization rates at three sites in Germany. Each point is the mean of the four replicates measured from March 2018 to February 2019 and from March 2019 to January 2020.

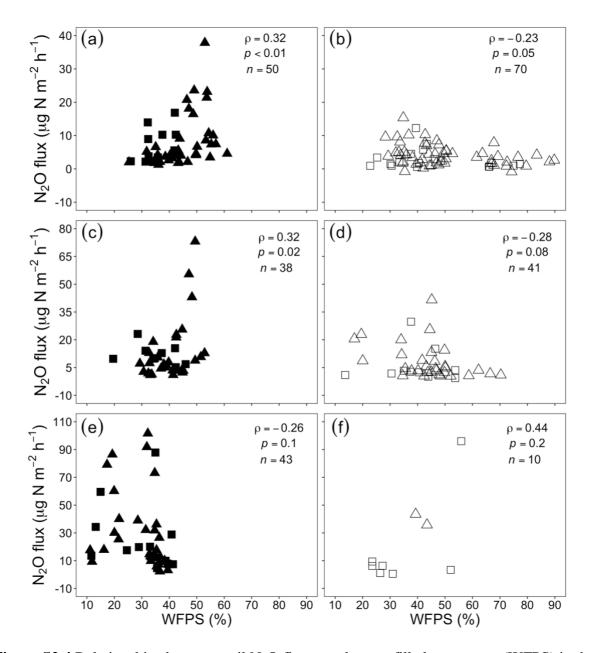


Figure S2.4 Relationships between soil N_2O fluxes and water-filled pore space (WFPS) in the top 5 cm depth in cropland agroforestry crop row and monoculture at three sites (a, b, Dornburg on loam Phaeozem soil; c, d, Wendhausen on clay Cambisol soil; e, f, Vechta on sandy Arenosol soil) in Germany. Spearman's rank correlation test was conducted separately during the cropping season (filled triangles for agroforestry crop row, filled squares for monoculture; a, c, e) and after crop harvest (open triangles for agroforestry crop row, open squares for monoculture; b, d, f). Each data point is the mean of the four replicates on each sampling day.

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Chapter 3

Changes in soil CO₂ and CH₄ fluxes after conversion of cropland monoculture to alley cropping agroforestry system

Guodong Shao, Guntars Martinson, Jie Luo, Xenia Bischel, Dan Niu, Marife D. Corre, Edzo Veldkamp

Soil Science of Tropical and Subtropical Ecosystems, Faculty of Forest Sciences and Forest Ecology, University, University of Goettingen, Goettingen, Germany

Abstract

Agroforestry is a promising management practice to increase carbon (C) sequestration and reduce greenhouse gas (GHG) emissions. In temperate Europe, agroforestry system is gaining increasing interests due to its potential for solving important environmental problems. However, the effects of agroforestry on the spatial-temporal dynamics of soil carbon dioxide (CO₂) and methane (CH₄) fluxes are presently poorly qualified. Here we present a systematic comparison of soil CO₂ and CH₄ fluxes between cropland agroforestry and monoculture systems, based on a two-year field measurement at three sites on different soils in Germany. Each site had adjacent alley cropping agroforestry system and monoculture, and the agroforestry was established on the former monoculture croplands 1 to 11 years prior to this study. We found that the areaweighted soil CO₂ emissions from agroforestry (3.5-8.1 Mg C ha⁻¹ yr⁻¹) were comparable to monocultures (3.4–9.8 Mg C ha⁻¹ yr⁻¹), while soil CH₄ uptake was increased by up to 300% in agroforestry (0.4–1.3 kg C ha⁻¹ yr⁻¹) than monocultures (0.1–1.2 kg C ha⁻¹ yr⁻¹). The seasonal variations of soil CO₂ and CH₄ fluxes were strongly regulated by soil temperature and moisture, and the spatial variations were dominantly influenced by soil texture. Based on our results, conversion of monoculture cropland to alley cropping agroforestry system exhibits a great potential for mitigating CH₄ emissions, and the unfertilized tree rows in agroforestry system provide an opportunity to increase C sequestration in the long run.

3.1. Introduction

Agriculture is a major contributor to the global anthropogenic emissions of greenhouse gas (GHG), which are associated with crop cultivation, livestock and land-use change (IPCC 2014). Improved agricultural management practices and more soil carbon (C) sequestration may effectively mitigate GHG emissions. Agroforestry, a land-use management that integrates trees with crops and/or animals on the same agricultural land, is commonly considered as one of the sustainable strategies to mitigate and adapt to climate change (Zomer et al., 2016). Compared with conventional agricultures, agroforestry systems can provide multiple ecosystem services and environmental benefits through increasing C sequestration (Peichl et al., 2006; Amadi et al., 2016), nutrient availability (Pardon et al., 2017), biodiversity (Banerjee et al., 2016; Beule and Karlovsky, 2021), and soil water use efficiency (Schwendenmann et al., 2010a). Moreover, introducing trees into arable lands may potentially increase soil organic carbon (SOC) content through continuous tree-litter input (Amadi et al., 2016; Pardon et al., 2017) and promote soil microclimate (e.g., temperature and moisture) stability (Quinkenstein et al., 2009;Gomes et al., 2016), which directly affect soil microbial activity. Thus, implementing agroforestry system offers an opportunity to reduce carbon dioxide (CO₂) and methane (CH₄) emissions from agricultural soil.

Soil respiration represents the second-largest C flux between terrestrial ecosystems and atmosphere (Hanson et al., 2000). Even small changes in soil respiration are likely to affect CO₂ concentration in the atmosphere and further impact global C cycle (Bahn et al., 2009). Soil CO₂ fluxes are the result of respiration processes from soil organic matter (SOM) decomposition by soil microbes (heterotrophic respiration) and roots (autotrophic respiration) (Hanson et al., 2000). These processes are primarily influenced by soil temperature, moisture, and substrate availability (Davidson et al., 2006; Gershenson et al., 2009; Meyer et al., 2018). It has been well illustrated that soil CO₂ emissions are positively correlated with temperature in both cropping and forest systems (Gauder et al., 2012; Luo et al., 2012; Wordell-Dietrich et al., 2020). Soil CO₂ emissions generally exhibit a parabolic relationship with soil moisture, with emissions increased under favorable moisture conditions and decreased when soils are very wet that may limit gas diffusion and/or CO₂ production (Koehler et al., 2009;

Franzluebbers et al., 2017; Tchiofo Lontsi et al., 2020). Earlier studies suggest that the spatial variability of soil CO₂ emissions can also be influenced by texture (Sotta et al., 2006; Hassler et al., 2015), substrate availability (Gershenson et al., 2009), vegetation type (Raich & Tufekcioglu, 2000), and land-use change (Edzo Veldkamp et al., 2020).

CH₄ is the second most important greenhouse gas contributor to climate change after CO₂, with a global warming potential 28–34 times greater than CO₂ on a 100-year timescale (IPCC, 2014). Global atmospheric concentration of CH₄ has increased from a pre-industrial level of 720 ppb to 1860 ppb in 2018 (Jackson et al., 2019). Soils act as a source or sink of CH4 depending on the balance between the production of CH4 by methanogenic microorganisms under anaerobic conditions and oxidation by methanotrophic microorganisms under wellaerated conditions (Le Mer & Roger, 2001). Thus, soil CH4 fluxes are strongly determined by controlling factors that can influence gas diffusion and soil microbial activity (Dobbie and Smith, 1996; Veldkamp et al., 2013; Gatica et al., 2020). Soil moisture is recognized as the most important factor that drives soil CH4 fluxes in the soil surface (Veldkamp et al., 2013; Matson et al., 2017; Martinson et al., 2021). Studies have shown the positive effect of soil temperature on soil CH₄ uptake (Gauder et al., 2012; Walter et al., 2015), since high temperature generally contribute to a lower soil water content as well as increased microbial activity, and thus stimulates CH₄ consumption in soils (Dobbie and Smith, 1996). Soil texture is another factor that influences CH4 fluxes (Veldkamp et al., 2013; Martins et al., 2017), depending on the especially important role of coarse pores in regulating the diffusion of atmospheric CH4 into the soil (Veldkamp et al., 2013). In addition, CH₄ production and consumption in soils could also be influenced by N availability (Veldkamp et al., 2013; Martinson et al., 2021) and pH (Borken et al., 2003).

In agroforestry systems, soil CO₂ and CH₄ fluxes can be influenced by the changed soil properties and nutrient availability (Amadi et al., 2016; Franzluebbers et al., 2017; Kim et al., 2016). Amadi et al. (2016) reported that soil CO₂ emissions were increased under trees or adjacent cropped fields, which probably contribute to the increased root respiration of trees during growing periods and enhanced SOC decomposition by litterfall input. However, introducing trees into croplands may also maintain (Medinski et al., 2015) or decrease

(Franzluebbers et al., 2017) soil CO₂ emissions depending on the stages of tree growth. In addition to the influence on soil CO₂ fluxes, agroforestry systems play a role in regulating the annual C budget by increasing both above- and belowground biomass stocks and enhancing C sequestration (Jose, 2009; Kim et al., 2016). Estimated net C balance for the agroforestry and sole cropping systems indicates the potential of agroforestry systems to act as C sink and to reduce atmospheric CO₂ concentration (Peichl et al., 2006). Soil CH₄ uptake under trees from agroforestry system can be enhanced as a result of the greater soil water uptake by tree roots (Amadi et al., 2017) and lower bulk density resulting from root activity (Amadi et al., 2016; Kim et al., 2016). Since trees in temperate agroforestry systems are generally not fertilized (Tsonkova et al., 2012; Schmidt et al., 2021), and thus may have higher CH₄ uptake compared to croplands, because of the inhibition effect of N fertilizer on CH₄ oxidation (Veldkamp et al., 2013). Therefore, quantifying the relationships of soil CO₂ and CH₄ fluxes with controlling factors in agroforestry and monoculture systems will provide us with a better understanding of the GHG dynamics.

In temperate Europe, a novel type of alley cropping seems to be a promising alternative compared to conventional agriculture. Presently, there is only one study has focused on the potential of alley cropping agroforestry systems for mitigation of CO₂ emissions from soil in Germany, based on a seven months measurement period (Medinski et al., 2015). The objectives of the present study were to (1) assess the changes in soil CO₂ and CH₄ fluxes after conversion of cropland monoculture to alley cropping agroforestry system, and (2) determine the temporal and spatial controls of soil CO₂ and CH₄ fluxes, based on a two-year field measurement at three sites on different soils in Germany. We hypothesized that (1) alley cropping agroforestry systems will have higher soil CO₂ and CH₄ fluxes will be regulated by soil moisture and temperature, soil CH₄ fluxes will increase with increasing mineral N availability; the spatial patterns of soil CO₂ and CH₄ fluxes will be regulated by soil texture. Our study provides a foundation for estimating the net carbon balance and GHG budgets after the conversion of cropland monoculture to agroforestry systems in temperate regions.

3.2. Materials and methods

3.2.1. Study sites and experimental design

Soil CO₂ and CH₄ fluxes were measured at three sites in Germany (Figure 2.1a): Dornurg (51°00'40" N, 11°38'46" E, Thuringia) with a loam Phaeozem soil (Table S2.1), Wendhausen (52°20'00" N, 10°37'55" E, Lower Saxony) with a clay Cambisol soil and Vechta (52°45'29" N, 8°32'05" E, Lower Saxony) with a sandy Arenosol soil, where cropland monocultures were converted into alley cropping agroforestry systems. During our measurement periods (March 2018 to February 2019 and March 2019 to January 2020) the accumulation precipitation was 445 mm and 494 mm on the loam Phaeozem soil, 379 mm and 539 mm on the clay Cambisol soil, 432 mm and 666 mm on the sandy Arenosol soil; the mean annual temperature was 11 °C at all sites (Table S2.1).

Each site had a cropland agroforestry that consisted of tree strips alternated with crop alleys and an adjacent cropland monoculture (Figure 2.1b-d). At the sites with loam Phaeozem and clay Cambisol soils, agroforestry was established on a monoculture cropland by planting 12-m wide rows of fast-growing poplar (clone Max1, Populus nigra \times P. maximowiczii) alternated with 48-m wide rows of crop alleys in 2007 and 2008, respectively (Figure 2.1b). The aboveground biomass of the tree row in agroforestry system was first harvested for bioenergy production in January 2015 at the site with loam Phaeozem soil and in January 2014 at the site with clay Cambisol soil. In addition, from an earlier study at our study sites on crops' nutrient response efficiency (NRE, measured in 2016 and 2017), both monoculture and agroforestry crop rows were at the nutrient saturation range in terms of fertilization rate and soil available nutrients (Schmidt et al., 2021). Thus, to compare the effects of reduced fertilizer input on soil CO₂ and CH₄ fluxes in agroforestry system, we established a newly paired cropland agroforestry and monoculture plots at the site with loam Phaeozem soil in March 2019. Besides receiving no fertilization, all the experimental design and management practices were the same for the additional agroforestry and monoculture systems. At the site with sandy Arenosol soil, agroforestry was established in April 2019 by planting a 12-m wide row of poplar in the middle of the field, alternating with 48-m wide rows of crop alleys at two aspects

of the tree row (Figure 2.1c).

At each site, we established four replicate plots in both cropland agroforestry and monoculture systems (Figure 2.1b–d). In agroforestry, each replicate plot was established with a transect perpendicular to the tree row and each transect consisted of four sampling locations: in the middle of the tree row, in the crop row at distances of 1 m, 7 m, and 24 m from the edge of the tree row (Figure 2.1b, c). In monoculture, sampling points were located in the center of each replicate plot (Figure 2.1d). In total, we had 20 sampling plots at each site (four replicate plots in the agroforestry × four sampling locations + four replicate plots in the monoculture). At each site, the agroforestry crop row received the same management practices as monoculture on the same date, the agroforestry tree row receive no fertilizer after planting (which is the common practice of our farmer collaborators; Schmidt et al., 2021). In the field, we observed that the fertilizer broadcaster drove at 12 m from the tree row; the fertilizers were broadcasted for the remaining 24 m crop row to be fertilized. At mid-way (24 m) of the agroforestry crop row, the fertilizers were broadcasted with about 1 m overlapped, such that at the middle of this crop row.

3.2.2. Soil CO₂ and CH₄ fluxes measurement

At the three sites with normal fertilization, we measured soil CO₂ and CH₄ fluxes at monthly intervals from March 2018 to January 2020; for the follow-on experiment received no fertilization at the site on loam Phaeozem soil, we measured soil CO₂ and CH₄ fluxes monthly from March 2019 to January 2020. For the measurement period from March 2018 to February 2019 at the site on sandy Arenosol soil, soil CO₂ and CH₄ fluxes were measured in pre-established eight sampling plots prior to the establishment of agroforestry in April 2019, and all replicate plots were still under monoculture. Due to logistic reasons, measurements in June or July 2018 (extreme drought in Germany), December 2018 and/or January 2019 (frozen soil) were unable to conduct at the three sites. In each sampling plot within each site, gas samples were measured with vented static chambers (e.g., Hassler et al., 2015; Matson et al., 2017; Tchiofo Lontsi et al., 2020) that consisted of chamber bases made of polyvinyl chloride (0.04

 m^2 in area, 0.24 total height, inserted into the soil ~0.03 m) and vented static, polyethylene chamber hoods (resulting in ~10.5 L total chamber volume), equipped with a Luer-lock sampling port for headspace gas sampling. In agroforestry crop rows and monocultures, we installed the chamber bases before the gas measurement started in the morning and removed them after sampling on each sampling day, in order to not hamper farmers' field activities. In agroforestry tree rows, we placed the chambers in each of the four sampling locations in the middle of the tree row before our first measurement at each site. From each chamber, gas samples (25 ml each) were collected four times over a 32-min period (at 2, 12, 22, and 32 min after chamber closure) using plastic syringes. The gas samples were immediately stored in pre-evacuated glass vials (Exetainers, 12 mL; Labco Limited, Lampeter, UK) with rubber septa that were only used once.

The concentrations of the gas samples were analyzed serially using a gas chromatograph (GC; SRI 8610C, SRI Instruments Europe GmbH, Bad Honnef, Germany), equipped with an auto-sampler and a flame ionization detector (with a methanizer for CO₂). For each analysis, we calibrated the GC using three calibration gases (Deuste Steininger GmbH, Mühlhausen, Germany) with concentrations ranging from 400 to 3000 ppm for CO₂ and 1000 to 5000 ppb for CH₄. Soil CO₂ and CH₄ fluxes were calculated from the linear change of the detected gas concentrations over time of chamber closure and adjusted with the simultaneously measured air temperature and pressure in the field during gas sampling. We regarded the linear increase of CO₂ concentrations with time as our reference for the quality check of CH₄ concentrations within each chamber. We observed all chamber measurements of the CO₂ concentrations increased linearly during the 32-minute chamber closure ($R^2 > 0.9$), justifying a linear fit was applied for both gases. For the data analyses of CH4, all measured fluxes (i.e. zero, positive and negative) were included in order to avoid bias. Annual soil CO2 and CH4 fluxes from each sampling location at each replicate plot were estimated using the trapezoidal interpolation between monthly measured fluxes and time intervals during March 2018-February 2019 and March 2019–January 2020 (the latter was ratioed to 365 days) (e.g., Hassler et al., 2015; Matson et al., 2017; Tchiofo Lontsi et al., 2020; Martinson et al., 2021).

To compare soil CO₂ and CH₄ fluxes between agroforestry and monoculture at the system

level, we calculated the overall values for agroforestry by weighting the areal coverage of the tree row and crop row sampling locations. The weighting factors were calculated by considering the widths of the crop row (24 m) and half of the tree row (6 m), totalling to 30 m, as the alternating tree and crop rows indicated that half of their widths represented each side of the rows (Figure 2.1b, c). Considering the 1-m width overlap of the fertilizer at the 24 m sampling location in the crop row (see section 2.1), we calculated the overall soil CO₂ and CH₄ fluxes for agroforestry using the weighting factors of the tree row (0.2, 6 m/30 m), 1 m (0.13, 4 m/30 m), 7 m (0.6, 18 m/30 m) and 24 m (0.07, 2 m/30 m). Thus, the equations (1) and (2) we used to calculate the weighted soil CO₂ and CH₄ fluxes for agroforestry system and agroforestry crop row as follows:

$$F_{AF} = (6 \times F_0 + 4 \times F_1 + 18 \times F_7 + 2 \times F_{24})/30 \tag{1}$$

$$F_{AFC} = (4 \times F_1 + 18 \times F_7 + 2 \times F_{24})/24 \tag{2}$$

where F_{AF} and F_{AFC} are CO₂ and CH₄ fluxes of agroforestry system and agroforestry crop row; F_0 , F_1 , F_7 , and F_{24} are CO₂ and CH₄ fluxes of sampling locations at the tree row, at 1 m, 7 m, and 24 m within the crop row; numbers 6, 4, 18, and 2 are the weighting coefficients of each sampling location.

3.2.3 Supporting soil factors

On each day of gas sampling, the potential soil controlling factors (i.e., temperature, WFPS and extractable mineral N) were measured concurrently in the top 0.05 m depth near each chamber base. Soil temperature was determined close to each chamber using a GMH 1170 digital thermometer (Greisinger electronic GmbH, Regen-stauf, Germany). At each sampling plot within each site, four soil samples were taken using diameter cylinders (250 cm³ in volume). One sample was transported to the laboratory and oven-dried at 105 °C for 24 h to determine the gravimetric moisture content and bulk density, which were calculated for WFPS using a theoretical particle density of 2.65 g cm⁻³ for the mineral soil. The remaining three soil cores were pooled and mixed thoroughly in the field, and a fresh soil sample (~50 g) was put into the pre-prepared bottles containing 150 ml 0.5 M K₂SO₄ for mineral N extraction. After returning to the laboratory at the University of Goettingen on the same day, the extraction

bottles were shaken for 1 h, filtered through 0.5 M K₂SO₄ pre-washed filter papers and kept frozen until analysis. The extractable mineral N (NH₄⁺, NO₃⁻) and total extractable N concentrations were analyzed using continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany), where NH₄⁺ was determined by salicylate and dicloroisocyanuric acid reaction method (Autoanalyzer Method G-102-93) and NO₃⁻ by cadmium reduction method with NH₄Cl buffer (Auto-analyzer Method G-254-02) and total extractable N by ultraviolet-persulfate digestion followed by hydrazine sulfate reduction.

The general soil physical and chemical parameters within the top 0.3 m (texture, pH, organic carbon, total N, and effective cation exchange capacity) were measured once using standard methods as described in our previous work (Schmidt et al., 2021; Table S2.2).

3.2.4 Statistical analysis

For the parameters measured monthly, we first checked the normal distribution (using Shapiro-Wilk test) and equality of variance (using Levene's test), and the data with non-normal distribution were used either a logarithmic (i.e., WFPS, NH4⁺ and NO3⁻) or a square root (i.e., CO₂) transformation. Linear mixed-effects (LME) models (Crawley, 2007) were applied to test the differences in soil CO₂ and CH₄ fluxes and soil parameters (temperature, WFPS, NH₄⁺ and NO₃⁻) between management systems (i.e., agroforestry sampling locations and monoculture) within each site and among sites for monoculture. For LME tests, either management system (when comparing agroforestry sampling locations and monoculture within each site) or site (when comparing monocultures among sites) were fixed effects and sampling days and replicate plots were random effects. To evaluate the differences in soil CO₂ and CH₄ fluxes between the whole agroforestry and the monoculture, the agroforestry was weighted by the areal coverage of the tree row and crop row sampling locations, and LME tests were conducted as above. Significant differences between management systems or among sites were assessed using the analysis of variance (ANOVA) with Fisher's least significant difference test. For the general soil physical and chemical characteristics measured once (Table S2.2), we tested the differences between management systems within each site using a one-way ANOVA followed by Tukey HSD test or Kruskal-Wallis test (variables were non-normally distributed).

To assess the temporal influence of soil variables (temperature and WFPS) on soil CO₂ and CH₄ fluxes, we carried out the linear and non-linear regressions using the mean values of four replicate plots (or eight replicate plots during the measurement period from March 2018 to February 2019 on the sandy Arenosol soil) on each sampling day and conducted the regression across management systems, and over the entire study period for each site. Considering that there would be autocorrelation between the soil variables, Pearson's correlation tests were conducted between soil CO₂ and CH₄ fluxes, soil temperature, WFPS, and extractable mineral N using the dataset described above. We further used Spearman's rank correlation test to assess the spatial control of the one-time measured soil physical and chemical characteristics on soil CO₂ and CH₄ fluxes, using the four replicates in cropland agroforestry tree row, crop row and monoculture at the sites with loam Phaeozem and clay Cambisol soils and eight replicates in monoculture at the site with sandy Arenosols soil, measured in 2018–2019 (from March 2018 to February 2019) and 2019–2020 (from March 2019 to January 2020). For all tests, statistical significances were considered at $P \le 0.05$. All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019).

3.3. Results

3.3.1. Soil CO₂ emissions

The monthly soil CO₂ emissions from agroforestry tree row, crop row and monoculture (Figure 3.1 and Figure S3.1a) generally increased with the increasing soil temperature (ranging from 5 to 24 °C; Figure S2.1a–c and Figure S2.2a) and the decreasing WFPS (ranging from 12 to 67 %; Figure S2.1d–f and Figure S2.2b) during spring and early summer (from March to July). In a few cases where soil CO₂ emissions declined sharply with WFPS in June 2018 and July 2019 at the site on loam Phaeozem soil, when the soil temperature was still high (Figures S2.1a, d and S2.2a, b). In the loam Phaeozem and clay Cambisol soils, soil CO₂ emissions decreased with the decreasing wFPS (ranging from 14 to 92 %; Figure S2.1d–f and Figure S2.2b) towards fall and winter (from August to February), whereas in the sandy Arenosol soil, soil CO₂ emissions increased with the increasing WFPS when soil temperature was decreasing during

July to October in 2019 (Figure S2.1c, f).

In the loam Phzeozem and clay Cambisol soils, CO₂ emissions did not differ between management systems in both measurement periods (P > 0.13; Table 3.1). In the sandy Arenosol soil, CO₂ emissions from agroforestry crop row and monoculture were comparable (P > 0.76; Table 3.1) whereas were larger than that from agroforestry crop row (P < 0.01; Table 3.1). Considering the comparison between the agroforestry as a whole (weighted by the areal coverages of the tree and crop rows) and the cropland monoculture, there were no differences in soil CO₂ emissions between the two systems at each site (P > 0.12; Table 3.1). Comparing the cropland monocultures, soil CO₂ emissions in the loam Phaeozem soil were comparable with those in the clay Cambisol and sandy Arenosol soils (P > 0.10; Table 3.1), whereas the sandy Arenosol soil had higher soil CO₂ emissions in the sandy Arenosol soil in 2018–2019 (P < 0.01; Table 3.1); in 2019–2020, soil CO₂ emissions in the sandy Arenosol soil were higher than those in the loam Phaeozem and clay Cambisol soils (P < 0.01; Table 3.1).

3.3.2 Soil CH₄ fluxes

The monthly CH₄ fluxes from agroforestry tree row, crop row and monoculture did not show any clear seasonal patterns (Figure 3.2 and Figure S3.1b). In the loam Phaeozem and clay Cambisol soils, the majority of CH₄ fluxes showed net uptake, despite some occasional emissions were detected (24 emission fluxes out of 265 plot-mean fluxes or 9% of the observations, ranging from 0.1 to 10.6 μ g C m⁻² h⁻¹), whereas in the Arenosol soil, all CH₄ fluxes were net uptake.

Over each experiment period, the soils in all three sites acted as CH₄ sinks (Table 3.1). In the loam Phaeozem soil, the mean soil CH₄ uptake did not differ between management systems in 2018–2019 (P = 0.52), whereas in 2019–2020, soil CH₄ uptake from the 1 m sampling location in the agroforestry crop row was higher compared to those from the other management systems (P < 0.05). In the follow-on experiment without fertilization on the loam Phaeozem soil, soil CH₄ uptake at 1 m was higher than at 7 m in the agroforestry crop row (P = 0.03). In the clay Cambisol soil, a similar pattern was observed like that in the loam Phaeozem soil in 2018–2019, when soil CH₄ uptake was comparable between the management systems (P =0.09); in 2019–2020, soil CH₄ uptake from the monoculture was lower than those from the 1 m and 24 m sampling locations in the agroforestry crop row (P < 0.01), and was comparable to those from the agroforestry tree row and at the 7 m sampling location in the agroforestry crop row (P > 0.19). In the sandy Arenosol soil in 2019–2020, there were no differences in soil CH₄ uptake between management systems (P = 0.23).

During the two measurement periods, soil CH₄ uptake did not differ between the entire agroforestry and the monoculture in the loam Phaeozem and sandy Arenosol soils (P > 0.14; Table 3.1). In the clay Cambisol soil, soil CH₄ uptake from the whole agroforestry was larger than from the monoculture in both measurement periods (P < 0.04). Among the cropland monocultures, the sandy Arenosol soil had the largest soil CH₄ uptake, followed by the loam Phaeozem and clay Cambisol soils (P < 0.03); in 2019–2020, the additional monoculture without fertilization had larger soil CH₄ uptake than the original monoculture in the loam Phaeozem soil (P = 0.03; Table 3.1).

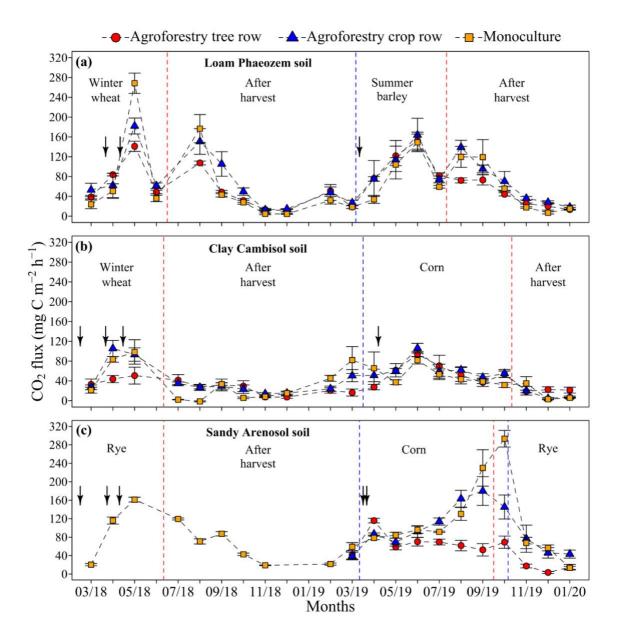


Figure 3.1 Monthly mean (\pm SE, n = 4 plots) soil CO₂ fluxes in cropland agroforestry tree row, crop row and monoculture at three sites in Germany. Soil CO₂ fluxes in agroforestry crop row were area-weighted average of the 1 m, 7 m and 24 m sampling locations. Black arrows indicate fertilizer application in the agroforestry crop row and monoculture only; tree rows were commonly unfertilized. Blue vertical lines indicate sowing; red vertical lines indicate harvest.

Table 3.1 Mean (\pm SE, n = 4 plots) soil CO₂ fluxes and CH₄ fluxes and annual soil CO₂ fluxes and CH₄ fluxes from cropland agroforestry and monoculture systems at three sites in Germany, measured monthly in 2018–2019 (from March 2018 to February 2019) and 2019/2020 (from March 2019 to January 2020).

Soil type /	Management	CO ₂ fluxes (mg C m ⁻² h ⁻¹)		CH4 fluxes (µg C m ⁻² h ⁻¹)		Annual CO ₂ fluxes (Mg C ha ⁻¹ yr ⁻¹)		Annual CH4 fluxes (kg C ha ⁻¹ yr ⁻¹)	
Study site	system								
		2018-2019	2019-2020	2018-2019	2019-2020	2018-2019	2019-2020	2018-2019	2019-2020
loam	Tree row	$57.5\pm13.4^{\rm a}$	64.6 ± 15.0^{a}	-6.5 ± 2.7^{a}	-11.5 ± 3.0^{a}	5.0 ± 0.2	5.7 ± 0.5	-0.7 ± 0.1	-1.0 ± 0.2
Phaeozem /	1 m crop row	76.9 ± 20.8^{a}	85.1 ± 16.1^{a}	-10.3 ± 4.4^{a}	$\textbf{-17.3} \pm 4.0^{b}$	6.9 ± 1.0	7.6 ± 0.4	-1.0 ± 0.1	-1.6 ± 0.2
Dornburg	7 m crop row	73.8 ± 20.6^a	76.1 ± 15.8^{a}	-8.9 ± 2.8^{a}	-7.4 ± 2.2^{a}	6.6 ± 0.9	6.8 ± 0.3	-0.8 ± 0.2	-0.8 ± 0.2
(normal	24 m crop row	$69.9 \pm 16.9^{\rm a}$	67.2 ± 13.5^{a}	-8.7 ± 3.3^{a}	-8.1 ± 2.6^{a}	6.2 ± 0.6	6.1 ± 0.2	-0.8 ± 0.2	-0.7 ± 0.0
fertilization)	Agroforestry	$70.7 \pm 17.4^{\rm A}$	$76.4 \pm 14.8^{\rm A}$	$\textbf{-8.6} \pm 1.8^{A}$	$\textbf{-9.6} \pm 1.8^{A}$	6.3 ± 0.7	6.7 ± 0.2	-0.8 ± 0.1	-0.9 ± 0.1
	Monoculture	68.6 ± 29.7^{aA}	63.6 ± 16.4^{aA}	$-8.2\pm3.1^{\mathrm{aA}}$	$\textbf{-6.8} \pm \textbf{4.3}^{aA}$	6.1 ± 0.2	5.7 ± 0.1	-0.8 ± 0.1	-0.5 ± 0.2
loam	Tree row		60.9 ± 14.5^{a}		-13.7 ± 3.4^{ab}		5.3 ± 0.7		-1.3 ± 0.2
Phaeozem /	1 m crop row		77.0 ± 19.6^{a}		$\textbf{-15.8} \pm 4.3^{b}$		7.0 ± 1.0		-1.5 ± 0.4
Dornburg	7 m crop row		47.9 ± 11.3^{a}		$-7.9\pm3.0^{\mathrm{a}}$		4.3 ± 0.5		-0.7 ± 0.1
(without	24 m crop row		65.0 ± 13.6^{a}		$\textbf{-11.6} \pm 3.5^{ab}$		5.8 ± 0.5		-1.2 ± 0.1
fertilization)	Agroforestry		$55.5\pm11.7^{\rm A}$		$-10.4 \pm 2.2^{\mathrm{A}}$		5.0 ± 0.3		-1.0 ± 0.1
	Monoculture		$59.3 \pm 14.0^{\mathrm{aA}}$		-12.4 ± 4.2^{abA}		5.3 ± 0.2		-1.2 ± 0.1

clay	Tree row	29.4 ± 7.0^{a}	43.1 ± 9.3^a	-4.2 ± 5.7^{a}	$\textbf{-3.0}\pm2.4^{ab}$	2.6 ± 0.3	4.0 ± 0.3	-0.4 ± 0.1	-0.3 ± 0.1
Cambisol /	1 m crop row	32.8 ± 6.8^a	58.2 ± 14.2^{a}	-3.6 ± 2.4^{a}	-6.3 ± 2.3^{bc}	2.9 ± 0.6	5.5 ± 0.5	$\textbf{-0.3}\pm0.1$	$\textbf{-0.6} \pm 0.1$
Wendhauser	n 7 m crop row	40.6 ± 11.9^{a}	44.7 ± 9.6^a	-6.8 ± 3.0^{a}	-4.6 ± 3.0^{abc}	3.8 ± 0.4	4.0 ± 0.1	-0.6 ± 0.1	$\textbf{-0.4} \pm 0.1$
	24 m crop row	43.0 ± 14.6^{a}	52.3 ± 9.3^a	-7.3 ± 4.1^{a}	$-7.7 \pm 1.3^{\circ}$	4.0 ± 0.3	4.7 ± 0.4	$\textbf{-0.8} \pm 0.1$	$\textbf{-0.7} \pm 0.2$
	Agroforestry	$37.3\pm9.6^{\rm A}$	$46.7\pm9.2^{\rm A}$	$\textbf{-5.9} \pm 1.7^{B}$	$-4.7\pm1.6^{\rm B}$	3.5 ± 0.2	4.3 ± 0.1	-0.5 ± 0.1	-0.4 ± 0.1
	Monoculture	35.6 ± 11.7^{aA}	43.5 ± 10.8^{aA}	$-2.7\pm3.9^{\mathrm{aA}}$	$-1.1 \pm 1.1^{\mathrm{aA}}$	3.4 ± 0.3	3.8 ± 0.2	-0.2 ± 0.1	-0.1 ± 0.1
sandy	Tree row		52.1 ± 10.5^{b}		-15.6 ± 2.3^{a}		4.7 ± 0.3		-1.3 ± 0.0
Arenosol /	1 m crop row		98.9 ± 16.9^{a}		$\text{-}15.0\pm2.5^{a}$		9.2 ± 0.5		-1.4 ± 0.1
Vechta	7 m crop row		$96.7\pm18.7^{\rm a}$		-12.2 ± 2.4^{a}		8.9 ± 0.2		-1.3 ± 0.1
	24 m crop row		$88.1\pm12.7^{\rm a}$		-12.6 ± 2.3^{a}		8.1 ± 0.4		-1.1 ± 0.1
	Agroforestry		$88.2\pm15.0^{\rm A}$		$-13.3\pm1.8^{\rm A}$		8.1 ± 0.2		-1.3 ± 0.1
	Monoculture	$72.2\pm20.3^{\dagger}$	109.2 ± 25.9^{aA}	$-13.8\pm2.4^{\dagger}$	$-13.2 \pm 3.1a^{A}$	6.3 ± 0.1	9.8 ± 0.6	-1.3 ± 0.1	-1.2 ± 0.1

Note: For each site, means with different lowercase letters indicate significant differences between the monoculture and sampling locations within the agroforestry system and different capital letters indicate significant differences between agroforestry (weighted by the areal coverage of the tree row and crop row sampling locations) and monoculture (Linear mixed effects model with Fisher's LSD test at $P \le 0.05$). Annual soil CO₂ fluxes and CH₄ fluxes were calculated using the trapezoidal rule between fluxes and time intervals during the measurement periods of 2018–2019 and 2019–2020.

[†]Measurements in 2018–2019 were conducted prior to agroforestry establishment and all replicate plots were still under monoculture, n

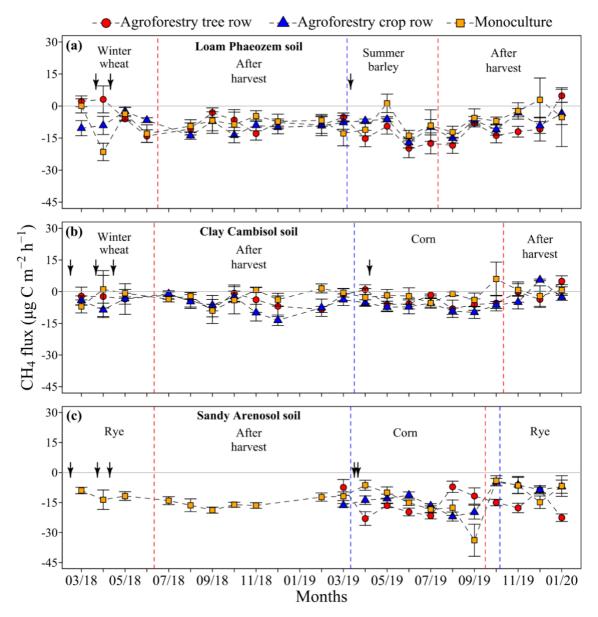


Figure 3.2 Monthly mean (\pm SE, n = 4) soil CO₂ fluxes (a) and CH₄ fluxes (b) in cropland agroforestry tree row, crop row and monoculture in loam Phaeozem soil (Dornburg site) without fertilizer application in 2019–2020. Blue vertical line indicates sowing; red vertical line indicates harvest.

3.3.3 Temporal and spatial controls of soil CO2 and CH4 fluxes

Over the two-year measurement period, the mean monthly soil CO₂ emissions from the agroforestry tree rows, crop rows and the monocultures showed a positive relationship with soil temperature within each site ($R^2 = 0.34-0.67$, P < 0.001; Figure 3.3a-c). In the loam Phaeozem and clay Cambisol soils, monthly soil CO₂ emissions displayed a parabolic relationship with WFPS (Figure 3.3d, e) and emissions during the warm seasons (April to September, with soil temperature ranging from 10 to 24 °C) were higher than those from the cold seasons (October to March, with soil temperature ranging from 0 to 10 °C; P < 0.01). In the sandy Arenosol soil, monthly soil CO₂ emissions were positively correlated with WFPS in the warm seasons when soil temperature was high whereas no correlation was detected in the cold seasons (Figure 3.3f). Although monthly soil CO₂ emissions showed a positive correlation with extractable mineral N in the clay Cambisol soil, this was not solely by mineral N but also by its auto-correlation with soil temperature (Table S3.1). Moreover, monthly soil CH4 fluxes were negatively correlated with soil temperature in the loam Phaeozem and sandy Arenosol soils (Figure 3.4a, c) and were positively correlated with WFPS at all sites (Figure 3.4d-f). We did not detect any significant correlations between soil CH₄ fluxes and the extractable mineral N (Table S3.1).

Across sites, the estimated annual CO₂ emissions during the two measurement periods were negatively correlated with the soil clay content, pH, total N and effective cation exchange capacity, and were positively correlated with bulk density (Table S3.2). Annual soil CH₄ fluxes showed opposing correlations with the above-mentioned soil characteristics because of the significant negative relationship between annual soil CO₂ emissions and CH₄ fluxes (Table S3.2).

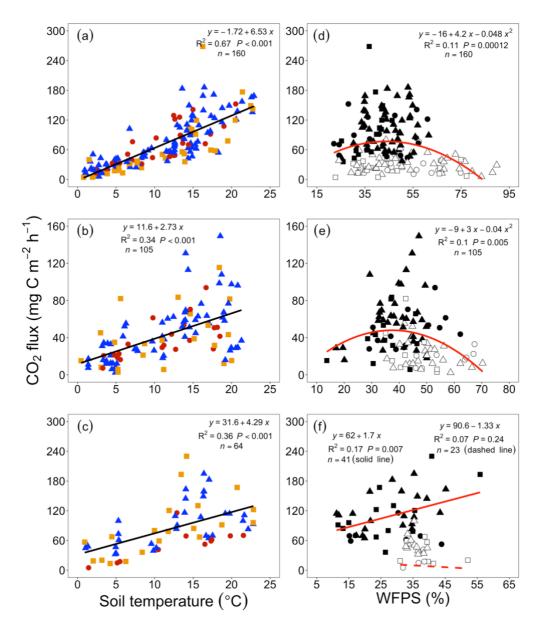


Figure 3.3 Relationships between soil CO₂ fluxes and soil temperature (a, b, c) and water-filled pore space (WFPS, d, e, f) in the top 5 cm depth in cropland agroforestry tree row, crop row and monoculture at three sites (a, d, Dornburg on loam Phaeozem soil; b, e, Wendhausen on clay Cambisol soil; c, f, Vechta on sandy Arenosol soil) in Germany. For the relationships between soil CO₂ fluxes and soil temperature: agroforestry tree row (•), crop row (\blacktriangle) and monoculture (\blacksquare). For the relationships between soil CO₂ fluxes and WFPS, regression analysis was conducted separately during the warm season: agroforestry tree row (•), crop row (\bigstar) and monoculture (\blacksquare), and cold season: agroforestry tree row (•), crop row (\bigstar) and monoculture (\square). Each data point is the mean of the four replicates on each sampling day.

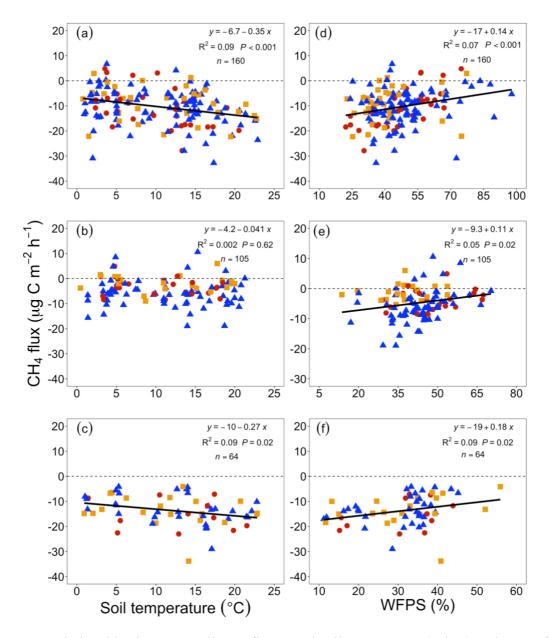


Figure 3.4 Relationships between soil CH₄ fluxes and soil temperature (a, b, c) and water-filled pore space (WFPS, d, e, f) in the top 5 cm depth in cropland agroforestry tree row, crop row and monoculture at three sites (a, d, Dornburg on loam Phaeozem soil; b, e, Wendhausen on clay Cambisol soil; c, f, Vechta on sandy Arenosol soil) in Germany. Agroforestry tree row (\bullet), crop row (\blacktriangle) and monoculture (\blacksquare). Each data point is the mean of the four replicates on each sampling day.

3.4. Discussion

3.4.1. Soil CO₂ emissions

Across sites, the annual soil CO₂ emissions from the poplar-based agroforestry tree rows during the two measurement periods were within the ranges reported for hybrid poplar and willow coppices soils (4.2–5.6 Mg C ha⁻¹ year⁻¹; Gauder et al., 2012; Kern et al., 2018), on the lower end range for beech forest soils (5.1–8.2 Mg C ha⁻¹ year⁻¹; Ngao et al., 2012; Rehschuh et al., 2019; Wordell-Dietrich et al., 2020), but lower than that found for spruce forest soil with high sand content (7.0–9.2 Mg C ha⁻¹ year⁻¹; Luo et al., 2012) in Germany. In comparison with other measurements from cropland in Germany, one study on a clay Luvisol soil that cropped with corn reported comparable annual soil CO₂ emissions (3.6–4.9 Mg C ha⁻¹ year⁻¹; Gauder et al., 2012) with our values from agroforestry crop rows and monocultures in the clay Cambisol soil, but lower than those values in the loam Phaeozem and sandy Arenosol soils, which may be related to the high clay content at that site compared to our sites.

At all sites, the similar seasonal patterns of soil CO₂ fluxes and soil temperature reflected the driving role of temperature on the production and release of CO₂ from the soil (Amadi et al., 2017). For example, increased soil temperature from spring to summer induced increasing soil CO₂ emissions, then both decreased towards fall, and reached the lowest in winter (Figure 3.1; Figure S2.1a–c). Similar findings were reported for the cropland (Gauder et al., 2012), forests (Luo et al., 2012; Wordell-Dietrich et al., 2020), and alley cropping system (Medinski et al., 2015a) in Germany and agroforestry systems in temperate North American regions (Amadi et al., 2017; Franzluebbers et al., 2017). The strong positive relationship between soil temperature and soil CO₂ emissions at each site highlights soil temperature was the most important controlling factor, this could be explained by the regulation of root respiration and microbial decomposition of SOM and plant residues by soil temperature (Luo et al., 2012). The greater soil CO₂ emissions during the warm periods (April to September) were attributed to the higher soil temperature increased root growth and turnover, as well as the microbial mineralization of SOM (Adviento-Borbe et al., 2010). The effect of soil moisture on soil CO₂ flux is generally variable. Under limited soil water conditions, the increasing soil moisture would increase root and microbial activities, and thus promote soil respiration (Koehler et al., 2009). For example, Tchiofo Lontsi et al. (2020) detected a positive correlation between WFPS and soil CO₂ emissions in a Congo Basin rainforest of Cameroon and Amadi et al. (2017) reported that the increasing soil moisture during summer periods strongly increased soil CO₂ emissions from a shelterbelt in Canada. However, we did not detect a linear relationship between the monthly WFPS and soil CO₂ emissions across management systems at each site. In fact, soil CO₂ emissions generally responded to changes in soil moisture on a seasonal scale (Koehler et al., 2009; Matson et al., 2017; Tchiofo Lontsi et al., 2020). In the sandy Arenosol soil, a positive relationship between WFPS and soil CO₂ emissions was observed when only considering the warm period (April to September; Figure 3.3f), which is probably attributed to the increasing soil water content enhanced microbial decomposition under high temperature conditions (Eric A. Davidson et al., 1998), suggesting soil CO₂ emission would be primarily regulated by soil moisture when CO₂ production was not limited by low soil temperature in the sandy texture dominated soils. The large interannual variation of soil CO₂ emissions from cropland agroforestry and monoculture systems in the clay Cambisol and sandy Arenosol soils (12–57%; Table 3.1), probably due to the frequent rainfall events during the warm seasons, which led to higher soil water content. The parabolic relationship between WFPS and soil CO2 emissions in the loam Phaeozem and clay Cambisol soils supported the strong influence of soil moisture on CO₂ emission. Similar relationship was also observed for an agroforestry system in southeastern USA (Franzluebbers et al., 2017), which reported greatest soil CO₂ emission occurred at the medium soil moisture and the high soil temperature levels. In the periods with high WFPS that result from snow melting in early spring and higher rainfall in winter, which limited oxygen diffusion into the soil and thus decreased microbial activity; while simultaneously, high soil moisture could have prevented CO₂ transport within and from the soil surface (Koehler et al., 2009; Tchiofo Lontsi et al., 2020). In addition, the seasonal patterns of soil CO₂ emissions from the mature agroforestry sites (i.e., loam Phaeozem and clay Cambisol) might be attributed to the positive correlations between soil CO₂ emissions and extractable mineral N. However, the relationship was not due to mineral N but also by its auto-correlation with soil moisture and temperature (Table S3.1).

Despite the mean soil temperature from the three sites being almost identical (Table 2.3), the annual soil CO₂ emissions were markedly different among the cropland monocultures (Table 3.1). The negative relationship between clay content and annual soil CO₂ emissions indicates the important role of soil texture in regulating CO₂ emissions. For example, higher annual soil CO₂ emissions were observed at the site with greater fine fraction soil texture (i.e., sandy Arenosol) compared to the sites with coarse texture (i.e., loam Phaeozem and clay Cambisol). The reason was probably due to higher sand content soil had lower ability to hold moisture and nutrients, which might enhance fine root turnover and thus increase root respiration (Sotta et al., 2006). Moreover, soils with higher clay content had the larger possibility that restricts the diffusion of CO₂ from the soil surface to atmosphere (Sotta et al., 2006; Hassler et al., 2015). Thus, the large variation in soil CO₂ emissions from monocultures suggests the spatial prediction of soil texture at the site scale.

3.4.2 Soil CH₄ fluxes

The average annual soil CH₄ uptake rates in the agroforestry tree rows were within the published ranges for poplar short rotation coppice (0.5–1.2 kg C ha⁻¹ year⁻¹; Walter et al., 2015) and within the lower end of the range reported for spruce forest (0.9–3.5 kg C ha⁻¹ year⁻¹; Luo et al., 2012), but lower than the values reported for beech forests (2.5–4.0 kg C ha⁻¹ year⁻¹; Rehschuh et al., 2019; Biernat et al., 2020) in Germany, which had sandy loam dominated soil textures. The studies reported annual soil CH₄ uptake rates from croplands in Germany (0–0.8 kg C ha⁻¹ year⁻¹; Gauder et al., 2012; Walter et al., 2015; Biernat et al., 2020) that were comparable with our values from agroforestry crop row and monoculture in the clay Cambisol soil, at the lower end of the ranges in the loam Phaeozem soil, but lower than the values in the sandy Arenosol soil, which may be attributed to the differences in clay content at each site. Among the cropland monocultures at our sites, the soil with coarse texture (i.e., sandy Arenosol) tend to have lower water retention capacity, resulting in higher gas diffusion and increasing the CH₄ availability for methanotrophs, and hence promoting higher soil CH₄ uptake compared to the soils with lower sand content (i.e., loam Phaeozem and clay Cambisol; Table 1). Moreover, the relationship between annual soil CH₄ fluxes and clay content supports the strong direct

effect of texture on CH₄ fluxes (Table S3.2).

Seasonal variation in soil CH₄ fluxes was strongly influenced by soil moisture with larger uptake when soil water content was generally low, which was reflected by the positive relationships between soil CH₄ fluxes and WFPS at all sites (Figure 3.4d-e). This is in line with the previous studies that have demonstrated soil moisture was the dominant controlling factor in soil CH₄ fluxes from forests (Veldkamp et al., 2013; Matson et al., 2017; Rehschuh et al., 2019; Tchiofo Lontsi et al., 2020) and cropping systems (Koga et al., 2004; Walter et al., 2015). The regulation of soil moisture on soil CH₄ uptake rates can be explained by influencing gas diffusivity (i.e., oxygen and CH₄) from the atmosphere into the soil, and further affect CH₄ oxidation and methanogenic activity (Keller and Reiners, 1994; Veldkamp et al., 2013; Martins et al., 2021). Even though we detected significant correlations between soil temperature and CH₄ fluxes in the loam Phaeozem and sandy Arenosol soils, the variation in CH₄ fluxes might not be explained by soil temperature but instead due to its indirect effect on soil moisture, which was reflected by the negative relationships between soil temperature and WFPS, and the negative relationships between soil CH₄ and CO₂ fluxes at our sites (Table S3.1). Moreover, Walter et al. (2015) and Martins et al. (2017) demonstrated that the positive influence of soil temperature on soil CH₄ uptake only occurred when gas diffusion is not limited by soil conditions. Contrary to our hypothesis, we did not observe any relationships between soil mineral N with soil CH₄ fluxes at all sites (Table S3.1). Similar observation was reported by Walter et al. (2015), where increased NH4⁺ content by fertilization had no effect on soil CH4 uptake from two sites cultivated with different bioenergy crops in Germany. Nonetheless, we found that the monoculture from the follow-on experiment received no fertilization had significantly higher soil CH₄ uptake than the original monoculture in the loam Phaeozem soil, similar findings of nitrogen fertilization inhibited soil CH4 uptake were also reported for temperate forests (Steudler et al., 1989) and cropping soils (Sainju et al., 2014). The reason can probably be explained by the fertilization induced more NH4⁺ competes with CH4 for the same active sites of methane monooxygenase, which leads to inhibition of CH4 oxidation (Bedard and Knowles, 1989; Steudler et al., 1989).

3.4.3 Effects of alley cropping agroforestry on soil CO2 and CH4 fluxes

To our knowledge, this study gives a first insight into the systematic comparison of soil CO₂ and CH4 fluxes from cropland agroforestry and monoculture systems in temperate regions. For the agroforestry system in the first year after establishment (i.e., sandy Arenosol soil), soil CO₂ emission from tree row was lower than the other managements, which caused a reduction in annual soil CO₂ emission by 17% from the whole agroforestry (area-weighted for tree and crop rows) than the monoculture (Table 3.1), suggesting the potential of young agroforestry system for mitigating soil CO₂ emissions. This is mainly associated with the smaller roots of young poplar trees, which highlights the large contribution of root respiration to total CO₂ emissions (Medinski et al., 2015). Despite heterotrophic respiration caused by plant residues and SOM decomposition is also an important source of total soil CO₂ flux, separation of heterotrophic emission from root respiration is not allowed based on our measurement period, since both components showed large seasonal variations throughout the year (Medinski et al., 2015; Franzluebbers et al., 2017). Therefore, further investigation is needed to quantify the relative contributions of root and heterotrophic CO₂ emissions to total soil respiration. At the sites with mature agroforestry systems (i.e., loam Phaeozem and clay Cambisol soils), we did not observe any significant differences in average soil CO₂ emissions between management systems (Table 3.1). This is in line with the findings that reported for a black locust and poplar based alley cropping system in Germany (Medinski et al., 2015), but contrary to the findings by Peichl et al. (2006) and Amadi et al. (2016, 2017), who reported soil CO₂ emissions under trees were higher than the adjacent cropped areas.

Indeed, our measured soil CO₂ emissions cannot be solely regarded as the indication for a net carbon sink or source of the agroforestry system. However, soil CO₂ emissions in the crop fields could be increased following annual fertilizer application, which resulted in higher root production and microbial activity (Amadi et al., 2016); in addition, long-term tillage practices during non-cropping seasons could reduce soil bulk density (Peichl et al., 2006) combined with plant residues input, which would also induce higher soil respiration by increasing gas diffusion and SOM mineralization (Medinski et al., 2015). Considering the tree rows in our sites occupied 20% of the agroforestry area, and were unfertilized since establishment and undisturbed by tillage, which may reduce fertilization-stimulated C loss and facilitate C sequestration in soil aggregates (Medinski et al., 2015) for the whole agroforestry system. Thus, we think that the alley cropping agroforestry system holds a great potential for increasing C sequestration in the long run.

The complicated interannual variation of soil CH₄ uptake within each site reflected that crop type may not directly affect CH₄ fluxes from soil, which was in line with the findings by Walter et al. (2015), who reported that CH₄ uptake is independent of the crop type. In the loam Phaeozem soil, the larger soil CH₄ uptake at 1 m location within the crop row in 2019–2020 probably related to the continuous litterfall input and decomposition, which might cause the soil bulk density turned to be lower, combined with the relatively lower soil moisture (Table 2.3), gas diffusion may then be enhanced and hence CH₄ uptake (Amadi et al., 2017). In the clay Cambisol soil, the monoculture presented the lowest soil CH4 uptake in 2019-2020 although it had the lowest WFPS (Table 2.3), which can be directly reflected by the frequent occurrence of CH4 emissions. Indeed, both CH4 oxidation and consumption processes in soil are biologically regulated by specialized microbial communities (Martins et al., 2017). Thus, when soil moisture reaches to levels above or below the optimum soil water content for methanotrophic activity, CH₄ uptake rates would be decreased (Luo et al., 2013; Martins et al., 2017). Additionally, the optimum soil water content for CH₄ uptake is site specific that depending on soil properties and soil moisture thresholds for controlling gas diffusion (Luo et al., 2013). Thus, we think the lower CH₄ uptake in monoculture resulted from the drought soil condition which inhibited methanotroph activity. In the sandy Arenosol soil, soil CH4 uptake showed no differences between management systems, indicating there was no short-term effect of conversion from cropland monoculture to agroforestry system on soil CH4 fluxes (Horemans et al., 2019). Across sites, the whole agroforestry increased soil CH₄ sink strength up to 300% compared to the monocultures (Table 3.1), which may be related to the regulation of trees on soil moisture in agroforestry crop rows, then influence gas diffusion and hence CH4 uptake.

Conclusions

This is the first study to compare soil CO₂ and CH₄ fluxes systematically from alley cropping agroforestry and monoculture systems, and it provides a unique dataset for estimating the net balance of carbon emissions after conversion of cropland monoculture to agroforestry systems in Central Europe. Our results showed that the seasonal variations of soil CO₂ and CH₄ fluxes were strongly regulated by soil temperature and moisture, and the spatial variations were mainly controlled by soil texture. Soil CH₄ uptake from agroforestry systems was increased by up to 300% compared to monocultures, which probably can be explained by the regulation of trees on soil moisture in agroforestry systems. Although we did not observe any differences in soil CO₂ emissions between cropland agroforestry and monoculture systems, the unfertilized tree rows still provide an opportunity to increase carbon sequestration in agroforestry systems in the long term. Following our results, additional studies are needed to estimate the net ecosystem carbon exchange and the microbial mechanisms of soil CO₂ and CH₄ dynamics at our sites.

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Appendix

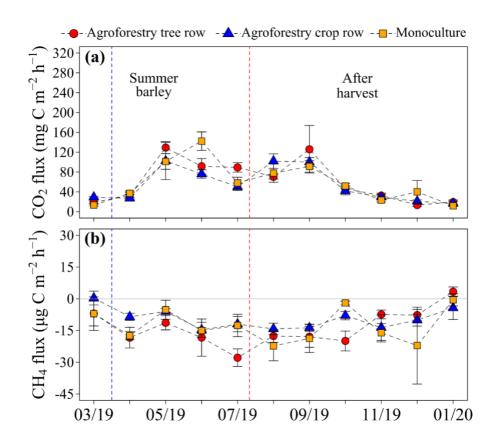


Figure S3.1 Monthly mean (\pm SE, n = 4) soil CO₂ fluxes (a) and CH₄ fluxes (b) in cropland agroforestry tree row, crop row and monoculture in loam Phaeozem soil (Dornburg site) without fertilizer application in 2019–2020. Blue vertical line indicates sowing; red vertical line indicates harvest.

Table S3.1 Pearson correlation coefficients between soil CO₂ fluxes (mg C m⁻² h⁻¹), soil CH₄ fluxes (μ g C m⁻² h⁻¹), soil temperature (°C), waterfilled pore space (WFPS), and extractable mineral N (NH₄⁺, NO₃⁻ and NH₄⁺ + NO₃⁻; mg N kg⁻¹), measured in the top 5 cm depth, across management systems at each site from March 2018 to January 2020.

Soil type / Study site	Soil variable	CH4 flux	temperature	WFPS	$\mathbf{NH4^{+}}$	NO ₃ -	Total mineral N
loam Phaeozem /	CO ₂ flux	-0.21*	0.83**	-0.17	0.24*	0.16	0.25*
Dornburg ($n = 105$)	CH ₄ flux		-0.25*	0.32**	0.02	-0.08	-0.01
(normal fertilization)	temperature			-0.36**	0.12	0.22*	0.17
	WFPS				-0.03	-0.28**	-0.12
loam Phaeozem /	CO ₂ flux	-0.51**	0.79**	-0.36**	0.44**	-0.12	0.20
Dornburg ($n = 55$)	CH4 flux		-0.42**	0.24	-0.04	-0.03	-0.05
(without fertilization)	temperature			-0.52**	0.40**	0.12	0.38**
	WFPS				-0.19	-0.35**	-0.44**
clay Cambisol /	CO ₂ flux	-0.15	0.56**	-0.23*	0.51**	0.32**	0.45**
Wendhausen ($n = 105$)	CH ₄ flux		-0.05	0.23*	0.02	-0.08	-0.06
	temperature			-0.56**	0.28**	0.48**	0.49**
	WFPS				-0.08	-0.25*	-0.23*
sandy Arenosol /	CO ₂ flux	-0.32**	0.56**	-0.05	-0.09	0.18	0.14
Vechta ($n = 64$)	CH4 flux		-0.29*	0.30*	0.14	-0.16	-0.11
	—						

temperature	-0.57**	0.11	0.65**	0.61**
WFPS		-0.14	-0.66**	-0.62**

Note: The correlation tests were conducted using the means of the four replicates on each sampling day, and thus *n* is the measurement days during the two-year study period.

* $P \le 0.05$, ** $P \le 0.01$.

Table S3.2 Spearman rank correlations of annual soil CO₂ fluxes (Mg C $ha^{-1} yr^{-1}$), soil CH₄ fluxes (kg C $ha^{-1} yr^{-1}$) and soil physical and biochemical characteristics across three sites in Germany.

	CH ₄ flux	Clay content	Bulk density	pН	Total N	Organic C	C: N ratio	Effective cation
								exchange capacity
CO ₂ flux	-0.71**	-0.64**	0.61**	-0.53**	-0.65**	-0.22	0.13	-0.55**
CH4 flux		0.69**	-0.67**	0.57**	0.59**	0.18	-0.18	0.59**
Clay content			-0.90**	0.89**	0.53**	-0.02	-0.48**	0.87**
Bulk density				-0.86**	-0.52**	0.00	0.47**	-0.89**
pН					0.37**	-0.15	-0.59**	0.97**
Total N						0.71**	0.19	0.43**
Organic C							0.77**	-0.08
C: N ratio								-0.55**

Note: The correlation tests were conducted using the four replicates in cropland agroforestry tree row, crop row and monoculture at the sites with loam Phaeozem and clay Cambisol soils and eight replicates in monoculture at the site with sandy Arenosols soil, measured in measured in 2018–2019 (from March 2018 to February 2019) and 2019–2020 (from March 2019 to January 2020), n = 76.

** $P \le 0.01$.

Chapter 4

Synthesis

4.1. Key findings of this thesis

1) Soil greenhouse gas fluxes from cropland agroforestry and monoculture systems

This research provides the first insight into the spatial effects of cropland agroforestry on soil greenhouse gas (GHG) fluxes from tree row and from crop row at distances of 1 m, 7 m and 24 m from the edge of the tree row, and provides a systematic comparison of GHG fluxes from cropland agroforestry and monoculture systems. In the agroforestry systems with mature (10–11 years old) trees, soil N₂O emissions were lowest in the tree rows and increased with distance into crop rows; soil CO₂ emissions did not differ among management systems; soil CH₄ uptake in the crop rows tended to be larger than the tree rows. In the agroforestry system with young (1-year-old) trees, soil N₂O emissions and CH₄ uptake did not differ between management systems, soil CO₂ emissions in the tree row were lower compared to the sampling locations in the crop row. Comparing between the entire agroforestry (weighted by the areal coverages of the tree and crop rows) and monocultures, soil N₂O emissions were comparable to monocultures; while soil CO₂ emissions from agroforestry were comparable to monocultures, while soil CO₂ emissions from agroforestry were comparable to monocultures, while soil CH₄ uptake from the whole agroforestry was increased by up to 300% than monocultures.

2) Effects of agroforestry on soil controlling factors

Although soil temperature in the tree rows was decreased up to 3 °C than in the tree rows and monocultures, there were no significant differences between management systems and among sites. At the sites with mature agroforestry systems, soil water-filled pore space (WFPS) was generally highest in the tree rows, followed by the crop rows and lowest in the monocultures. The reason was probably related to the reduction of wind speed in the agroforestry systems

compared to the monocultures (Böhm et al., 2014; Swieter et al., 2019), and therefore reducing soil evaporation, which consequently, decreasing water losses in the systems (Lin, 2010). Due to the lacking of fertilization (Schmidt et al., 2021), tree rows had the lowest soil extractable mineral N compared to crop rows and monocultures; soil mineral N in the crop rows showed an increasing trend from 1m to the 24 m sampling locations, which may be associated with the root competition between trees and crops (Allen et al., 2004; Amadi et al., 2017); At the site with young agroforestry systems, soil mineral N and WFPS did not differ between management systems.

3) Environmental factors controlling soil greenhouse gas fluxes

Our results showed that soil N₂O emissions were predominantly controlled by soil mineral N in both agroforestry and monoculture systems. Although no relationship was detected between soil N₂O fluxes and WFPS across the measurement period, when separating the influence of WFPS during the cropping seasons, a positive relationship between soil N₂O emission and WFPS at the sites with mature agroforestry systems, indicating soil moisture acts as a limiting factor under N-sufficient conditions. Soil temperature was the most important controlling factor for soil CO₂ emissions. The parabolic relationship between soil CO₂ emissions and WFPS in the loam Phaeozem and clay Cambisol soils, and the positive relationship between WFPS and soil CO₂ emissions during the warm periods (April to September) in the sandy Arenosol soil, suggesting soil CO₂ emission would be primarily regulated by soil moisture when CO₂ production was not limited by low soil temperature. Soil moisture was the dominant controlling factor in soil CO₂ and CH₄ fluxes were strongly regulated by texture.

4.2. Carbon budget implication and net global warming potential

Regardless of management practice and soil type, both agroforestry and monoculture systems evaluated in this study were net sources of N₂O and CO₂ sources and net sink of CH₄. At our sites, soil CO₂ emissions from the whole agroforestry (weighted by the areal coverages of the tree and crop rows) were increased by 3–18% than the monocultures at the sites with mature agroforestry systems, whereas were reduced by 17% at the site with young agroforestry system

(Table 3.1). However, our measured soil CO₂ emissions cannot be solely regarded as the indication for a net carbon (C) sink or source of the agroforestry system, because we did not measure the net amount of C uptake by trees and crops. Here we estimate the combined non-CO₂ GHG (N₂O and CH₄) emissions from cropland agroforestry and monoculture systems, we calculated non-CO₂ global warming potential (GWP) for each system within each site, using the CO₂-equivalents conversion factors of 265 for N₂O and 28 for CH₄ on a 100-year time scale (IPCC, 2014). Across sites, average annual soil non-CO2 GWP from agroforestry and monocultures systems ranged from 0.01–0.29 Mg CO_2 eq. ha⁻¹ yr⁻¹ and 0.03–0.33 Mg CO_2 eq. ha-1 yr-1, respectively (Table 4.1). At the sites on Clay Cambisol, sandy Arenosol, and unfertilized loam Phaeozem soils, the monocultures had higher GWP compared to agroforestry, whereas on the normal fertilized loam Phaeozem soil, agroforestry showed equal or higher soil N₂O emissions than monoculture, probably resulting from the fertilizers were broadcasted with about 1 m overlapped at the 24 m sampling location within crop row. Annual soil CH4 uptake showed almost no difference between the two systems. Over the two measurement periods, the total non-CO₂ GWP from agroforestry system was reduced by 0.22 Mg CO₂ eq ha⁻¹ compared to monocultures, highlighting the potential of agroforestry in mitigating soil GHG emissions from croplands.

Previous studies have demonstrated that conversion of cropland to agroforestry contributes to increase C sequestration and reduce net GHG emissions (Amadi et al., 2016; Kim et al., 2016). We further estimated net ecosystem GWP for both cropland agroforestry and monoculture systems as the net ecosystem C exchange + harvest C export (i.e. tree or crop biomass for bioenergy production, crop yield) + annual soil N₂O + CH₄ fluxes (Meijide et al., 2020). The net ecosystem C exchange (NEE) was estimated as net primary production (NPP) – soil heterotrophic respiration (Malhi et al., 1999). As we only measured the data of aboveground biomass (trees and crops), the belowground biomass was estimated according to the reported root/shoot ratios for the poplar trees and each crop type during our measurement period (Fortier et al., 2015; Hirte et al., 2018; Truan, et al., 2018). As discussed in Chapter 3, separation of heterotrophic respiration caused by plant residues and soil organic matter decomposition was not possible in the current study, soil heterotrophic respiration under trees

Table 4.1 Net global warming potential (GWP) of annual (\pm SE, n = 4) soil N₂O and CH₄ emissions from cropland agroforestry and monoculture systems at three sites in Germany, measured in 2018–2019 (from March 2018 to February 2019) and 2019–2020 (from March 2019 to January 2020).

Soil type / Study site	System	N2O (Mg CO2 eq. ha ⁻¹ yr ⁻¹)		CH4 (Mg CO ₂	eq. ha ⁻¹ yr ⁻¹)	Average Non-CO ₂ GWP	
		2018-2019	2019-2020	2018-2019	2019–2020	(Mg CO ₂ eq. ha ⁻¹ yr ⁻¹)	
loam Phaeozem / Dornburg	Agroforestry	0.10 ± 0.03	0.05 ± 0.00	$\textbf{-0.01} \pm 0.00$	$\textbf{-0.01} \pm 0.00$	0.06 ± 0.02	
(normal fertilization)	Monoculture	0.10 ± 0.01	0.04 ± 0.00	$\textbf{-0.01} \pm 0.00$	$\textbf{-0.01} \pm 0.00$	0.06 ± 0.01	
loam Phaeozem / Dornburg	Agroforestry		0.02 ± 0.01		$\textbf{-0.01} \pm 0.00$	0.01 ± 0.00	
(without fertilization)	Monoculture		0.04 ± 0.01		$\textbf{-0.01} \pm 0.00$	0.03 ± 0.01	
clay Cambisol /	Agroforestry	0.08 ± 0.02	0.15 ± 0.02	-0.005 ± 0.00	-0.004 ± 0.00	0.11 ± 0.01	
Wendhausen	Monoculture	0.06 ± 0.01	0.33 ± 0.05	$\textbf{-0.002} \pm 0.00$	$\textbf{-0.001} \pm 0.00$	0.19 ± 0.03	
sandy Arenosol /	Agroforestry		0.31 ± 0.05		$\textbf{-0.02} \pm 0.00$	0.29 ± 0.05	
Vechta	Monoculture		0.34 ± 0.04		-0.02 ± 0.00	0.33 ± 0.04	

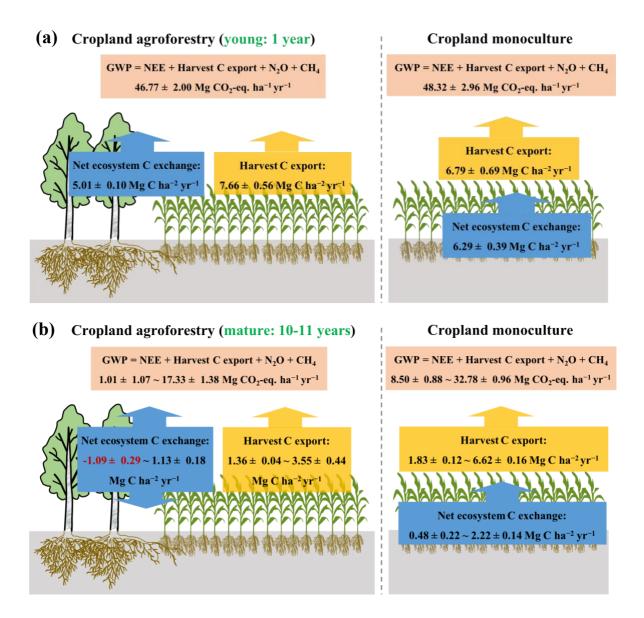


Figure 4.1 Mean annual carbon budget and net ecosystem global warming potential (GWP) estimated from the young (1 year old; a) and mature (10–11 years old; b) cropland agroforestry and monoculture systems. Negative and positive fluxes indicate sink and source, respectively.

and crops was estimated by the ratios of heterotrophic respiration/total soil respiration from previous studies (Verlinden et al., 2013; Zhang et al., 2013). At the site with young agroforestry system (Figure 4.1a), the NEE fluxes indicate that both agroforestry and monoculture systems were C source, but the NEE in agroforestry was lower and the harvest C export was higher than the monoculture; the combined net GWP showed that there was almost no difference between cropland agroforestry and monoculture systems. At the site with mature agroforestry systems (Figure 4.1b), the NEE fluxes showed that agroforestry systems act both C sink and source, whereas the monoculture was only a C source; the combined net GWP of agroforestry was much lower compared to the monoculture. Overall, our results highlight the great potential of agroforestry in increasing C sequestration and mitigating net GHG emissions.

4.3. Outlook

The research highlights that the conversion of monoculture cropland to agroforestry systems will affect soil GHG dynamics and further influence regional and global GHG budgets. Our results show the potential for reducing non-CO₂ GHG emissions and thus provide evidence for supporting policy implementation of agroforestry practices in temperate regions. Although the establishment of agroforestry had no effects on soil CO2 emissions compared to monocultures, considering the tree row in our sites occupied 20% of the agroforestry area, and were unfertilized since establishment and undisturbed by tillage, which may reduce fertilizationstimulated C loss and facilitate C sequestration in soil aggregates (Medinski et al., 2015) for the whole agroforestry system. In addition, since soil N₂O emissions were predominantly controlled by mineral N, our findings suggest that improved system management (e.g. optimal adjustments of the areal coverages between tree and crop rows) and optimized fertilizer input will enhance the potential of agroforestry for increasing carbon sequestration and mitigating net GHG emissions in the long term. Studies on our sites have shown the ability of cropland agroforestry to display nutrient saturation (Schmidt et al., 2021) and to decrease annual gross N₂O emission, and increase annual gross N₂O uptake (Luo et al., 2022). Following our results, further longer-time studies are needed to estimate the net ecosystem C exchange and the microbial mechanisms of soil GHG dynamics in the cropland agroforestry and monoculture systems.

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THESIS DECLARATION

I, Guodong Shao, hereby declare that I have completed all the four chapters of this dissertation entitled "Soil greenhouse gas (N₂O, CO₂ and CH₄) fluxes from cropland agroforestry and monoculture systems" by myself, and all references and data sources have been appropriately acknowledged. I have neither, nor will I, accept unauthorised outside assistance either free of charge or subject to a fee. I furthermore declare that this work has not been submitted elsewhere in any form as part of another dissertation procedure.

Göttingen, April 2022

(Guodong Shao)

CURRICULUM VITAE

Guodong Shao

Gender: Male Date of birth: 08.12.1989 Nationality: Chinese

EDUCATION



09.2010 - 07.2014 China Agricultural University, China

B. S. in Agronomy, Facility Agricultural Science and Engineering

09.2014 - 07.2017 Chinese Academy of Forestry, China

M. S. in Agronomy, Soil Science

12.2017 - 01.2022 Georg-August-Universität Göttingen, Germany

PhD candidate, Soil Science

PUBLICATIONS

1. **Shao, G.D.**, Martinson, G., Luo, J., Bischel, X., Niu, D., Corre, M.D., Veldlamp, E., (2022). Impacts of monoculture cropland to alley cropping agroforestry conversion on soil N₂O emissions. (*In prep.*).

2. **Shao, G.D.**, Martinson, G., Luo, J., Bischel, X., Niu, D., Corre, M.D., Veldlamp, E., (2022). Changes in soil CO₂ and CH₄ fluxes after conversion of cropland monoculture to alley cropping agroforestry system in Germany. (*In prep.*).

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PARTICIPATION AT CONFERENCES

02.2019 BonaRes Status Seminar in Leipzig, Germany

Poster presentation: Soil CO₂, N₂O and CH₄ fluxes from cropland agroforestry and monoculture systems

02.2020 BonaRes Status Seminar in Leipzig, Germany

Poster presentation: Soil CO₂, N₂O and CH₄ fluxes from cropland agroforestry and monoculture systems with reduced fertilization

06.2021 BonaRes Status Seminar (virtual), Germany

Poster presentation: Soil CO2 emissions from cropland agroforestry and monoculture systems

04.2021 **European Geosciences Union General Assembly 2021** (vEGU21: Gather Online) *Poster presentation*: Soil nitrous oxide emissions from temperate cropland agroforestry and monoculture system