



# **Effect of organic soil amendments on increasing soil N retention and reducing N losses from agricultural soils**

Zhijie Li

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Korreferent: Prof. Dr. Thomas Döring

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## Abstract

Loss of soil N through nitrous oxide ( $\text{N}_2\text{O}$ ), ammonia ( $\text{NH}_3$ ) emissions, and nitrate ( $\text{NO}_3^-$ ) leaching is considered to be the main pathways leading to environmental pollution. Applying high carbon amendments (HCA) may mitigate  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching. After HCA addition, soil microbes are stimulated to immobilize excess mineral nitrogen (N) from the soil, which is running at risk of getting lost in gaseous or dissolved form, to maintain the microbial C:N ratio of about 7. Moreover, the effect of carbon (C) availability on soil N content is moderated by phosphorus (P) availability, but so far, this aspect has not been adequately studied. Therefore, this thesis aimed to investigate the potential response patterns of soil N retention to changes in soil C, N, and P availability.

Chapter 2 used a meta-analysis to explore the effect of HCA on soil N retention at different locations, and for different climatic and soil conditions, and agricultural management strategies. On average, HCA incorporation stimulated  $\text{N}_2\text{O}$  emissions significantly by 29.7% but decreased  $\text{NO}_3^-$  leaching by 14.4%.

Chapter 3 investigated the impact of HCA on soil N dynamics at different P levels and soil types ((nutrient-rich silty soil (RUS), nutrient-rich sandy soil (RSS), and nutrient-poor silty soil (PUS)). The results revealed that the effect of HCA on PUS was not significant. Compared with leonardite, wheat straw and sawdust significantly increased  $\text{CO}_2$  emission and microbial biomass C in RUS, implying that the initial soil nutrient status is the determining factor for HCA degradation, and N dynamics in soil are strongly controlled by C and P availability.

Chapter 4 was an incubation experiment with a factorial design of one N level  $\times$  two P levels  $\times$  six C amendments. The added C amount in the form of  $^{13}\text{C}$ -glucose was 20% of the total C content of HCA. Compared with the control, HCA addition increased the microbial biomass C and N but decreased the  $\text{NO}_3^-$  content in the soil. The  $\delta^{13}\text{C}$  of the microbial biomass ( $\delta^{13}\text{C}$ -MB) and  $^{13}\text{C}$  recovery in MBC showed an increasing trend. HCA decreased the  $\delta^{13}\text{C}$ -MB, while P addition had the opposite effect. Hence, the labile glucose C was more readily available to microbes and probably formed stable substances that remain in the microbial community for a long time rather than being lost rapidly.

Chapter 5 presents data of a two-year lysimeter experiment. Compost was applied to assess the effect of nutrient-rich HCA on soil N retention and crop yield at different P levels. Compost application significantly increased plant aboveground biomass and grain yield, particularly in the double compost treatment, which increased winter wheat and winter barley yield by 62.4% and 34.8%, respectively. Double compost addition increased dissolved organic carbon and soil nutrient content, especially total N and P-CAL, while it caused no significant increase in greenhouse gas (GHG) emissions.

This work contributes to understanding how changes in soil C and P availability control soil N retention and crop yield, and can make a science-based recommendation that the combination of compost and mineral N fertilizer can increase crop yield without increasing GHG emissions and leaching of  $\text{NO}_3^-$  and P.

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## Zusammenfassung

Der Verlust von Stickstoff im Boden durch die Emission von Lachgas ( $\text{N}_2\text{O}$ ) und Ammoniak ( $\text{NH}_3$ ) und die Versickerung von Nitrat ( $\text{NO}_3^-$ ) gilt als Hauptursache für die Belastung der Umwelt mit reaktiven Stickstoffverbindungen. Der Einsatz von Bodenzusatzstoffen mit hohem Kohlenstoffgehalt (HCA) kann die  $\text{N}_2\text{O}$ -Emissionen und die  $\text{NO}_3^-$ -Auswaschung vermindern. Um das mikrobielle C:N-Verhältnis von etwa 7 aufrechtzuerhalten, werden Bodenmikroben nach der Zugabe von HCA angeregt, überschüssigen mineralischen Stickstoff (N) aus dem Boden zu immobilisieren, der in gasförmiger oder gelöster Form verloren zu gehen droht. Darüber hinaus wird die Auswirkung der Verfügbarkeit von Kohlenstoff (C) auf den Stickstoffgehalt des Bodens durch die Verfügbarkeit von Phosphor (P) beeinflusst, doch wurde dieser Aspekt bisher noch nicht ausreichend untersucht. Ziel dieser Arbeit ist es daher, die möglichen Reaktionsmuster der N-Retention auf Veränderungen der C-, N- und P-Verfügbarkeit im Boden zu untersuchen.

In Kapitel 2 wurde eine Meta-Analyse durchgeführt, um die Auswirkungen von HCA auf die N-Retention im Boden an verschiedenen Standorten, unter verschiedenen Klima- und Bodenbedingungen und mit verschiedenen landwirtschaftlichen Bewirtschaftungsstrategien zu untersuchen. Im Durchschnitt stimulierte die Einarbeitung von HCA die  $\text{N}_2\text{O}$ -Emissionen signifikant um 29.7 %, verringerte aber die  $\text{NO}_3^-$ -Auswaschung um 14.4 %. In Kapitel 3 wurden die Auswirkungen von HCA auf die N-Dynamik des Bodens bei verschiedenen P-Gehalten und Bodentypen (nährstoffreicher Schluffboden (RUS), nährstoffreicher Sandboden (RSS) und nährstoffarmer Schluffboden (PUS)) untersucht. Die Ergebnisse zeigten, dass die Wirkung von HCA auf PUS nicht signifikant war. Im Vergleich zu Leonardit erhöhten Weizenstroh und Sägemehl die Kohlendioxidemission und den mikrobiellen Biomasse-C-Gehalt in RUS erheblich, was bedeutet, dass der anfängliche Nährstoffstatus des Bodens der entscheidende Faktor für den HCA-Abbau ist und die N-Dynamik im Boden stark von der Verfügbarkeit von C und P gesteuert wird. Bei Kapitel 4 handelt es sich um einen Inkubationsversuch mit einem faktoriellen Versuchsplan von einer N-Stufe  $\times$  zwei P-Stufen  $\times$  sechs verschiedenen C-Zusätzen. Die zugesetzte C-Menge von  $^{13}\text{C}$ -Glucose betrug 20 % des gesamten C-Gehalts der HCA. Im Vergleich zur Kontrollgruppe erhöhte die HCA-Behandlung den C- und N-Gehalt der mikrobiellen Biomasse, verringerte jedoch den  $\text{NO}_3^-$ -Gehalt im Boden. Der  $^{13}\text{C}$ -Gehalt der mikrobiellen Biomasse ( $\delta^{13}\text{C}$ -MB) und die  $^{13}\text{C}$ -Wiederfindung in MBC zeigten einen steigenden Trend. HCA verringerte den  $\delta^{13}\text{C}$ -MB, während die Zugabe von P den gegenteiligen Effekt hatte. Daraus kann geschlossen werden, dass das labile C der Glukose für die Mikroben leichter verfügbar war und wahrscheinlich stabile Substanzen bildete, die lange in der mikrobiellen Gemeinschaft verbleiben, anstatt schnell verloren zu gehen. Kapitel 5 präsentiert Daten eines zweijährigen Lysimeterversuchs, in dem Kompost ausgebracht wurde, um die Auswirkungen von nährstoffreichem HCA auf die N-Retention im Boden und den Ernteertrag bei unterschiedlichen P-Gehalten zu untersuchen. Die Ausbringung von Kompost erhöhte die oberirdische Biomasse der Pflanzen und den Kornertrag erheblich, insbesondere bei der Behandlung mit der doppelten Menge Kompost, die den Ertrag von Winterweizen und Gerste um 62.4 % bzw. 34.8 % steigerte. Die Doppelkompostvariante erhöhte den gelösten organischen Kohlenstoff und den Nährstoffgehalt des Bodens, insbesondere den Gesamt-N und P-CAL, während sie keinen signifikanten Anstieg der Treibhausgasemissionen verursachte. Diese Arbeit trägt dazu bei zu verstehen, wie Veränderungen in der C- und P-Verfügbarkeit des Bodens die N-Speicherung im Boden und den Ernteertrag steuern, und kann eine wissenschaftlich fundierte Empfehlung abgeben, dass die Kombination von Kompost und

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mineralischem Stickstoffdünger den Ernteertrag steigern kann, ohne die Treibhausgasemissionen und die Auswaschung von  $\text{NO}_3^-$  und P zu erhöhen.

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## List of Abbreviations

AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
ATP	adenosine triphosphate
BD	bulk density
C	carbon
C:N	carbon-nitrogen ratio
C:N:P	carbon-nitrogen-phosphorus ratio
CaCO <sub>3</sub>	calcium carbonate
CEC	cation exchange capacity
CH <sub>4</sub>	methane
CO <sub>2</sub>	carbon dioxide
DD	decimal degrees
DMS	degrees/minutes/seconds
DNA	deoxyribonucleic acid
DNRA	dissimilatory nitrate reduction to ammonium
DOC	dissolved organic carbon
ECD	electron capture detector
FID	flame ionization detector
FTIR	Fourier-transform infrared spectroscopy
G	glucose
GC	gas chromatography
GHG	greenhouse gas
GWP	global warming potential
HCA	high organic carbon amendments
H <sub>2</sub> O	water
IPCC	Intergovernmental Panel on Climate Change
MAT	mean annual temperature
MAP	mean annual precipitation
MBC	microbial biomass carbon
MBN	microbial biomass nitrogen
N	nitrogen
N:P	nitrogen-phosphorus ratio
N <sub>2</sub>	nitrogen gas
N <sub>2</sub> O	nitrous oxide
NH <sub>3</sub>	ammonia

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$\text{NH}_4^+$	ammonium
NO	nitric oxide
$\text{NO}_2^-$	nitrite
$\text{NO}_3^-$	nitrate
NOB	nitrite-oxidizing bacteria
$\text{O}_2$	Molecular oxygen (dioxygen)
$\text{O}_3$	ozone
P	phosphorus
P-CAL	extract plant-available phosphorus
SIC	soil inorganic carbon
SOC	soil organic carbon
SOM	soil organic matter
TN	total nitrogen
TOC	total organic carbon
WHC	water holding capacity
$\delta$	isotope ratio of a sample or substance relative to an isotope ratio of a standard
$\delta^{13}\text{C}$	$^{13}\text{C}/^{12}\text{C}$ isotopic ratio of a sample relative to a standard





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## **Chapter 1**

### **Introduction**

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## 1.1 Background

As one of the dominant elements in nature, nitrogen (N) is widely involved in biological activities and is closely related to crop productivity, soil quality, and climate change. Since the invention of the industrial Haber-Bosch method for fixing  $N_2$  to ammonium ( $NH_4^+$ ), the problem of N deficiency in agriculture was solved. As a result, the N inputs to the biosphere have increased from 155 to 345 Tg year<sup>-1</sup>, in which synthetic N fertilizer is the main contributor (Bouwman et al., 2013). The global N demand for crop production in 2015 was 110 million tons and increased to nearly 119 million tons in 2020 (Lu and Tian, 2017). In addition, the Food and Agriculture Organization (FAO) estimates that in 2050 global meat production will increase to 455 million tons (Alexandratos and Bruinsma, 2012). Hence, farmers need to increase the N application amount to fulfill the growing demand for increasing crop yields. However, due to variations in soil texture, crop, climatic conditions, and overfertilization, only 10-65% of applied N is taken up by the crops (Liang et al., 2019; Zhang et al., 2011), and N fertilization rate is usually negatively correlated with N use efficiency (Cassman et al., 1998). Therefore, although soil microorganisms can retain soil nutrients, N in excess of plant requirements is an important factor of environmental pollution, leading to global warming, water eutrophication, and soil acidification. In addition, it may lead to the loss of humus and biological functions to regulate nutrient availability (Kosolapova et al., 2016).

The most important pathways of N loss are by nitrous oxide ( $N_2O$ ) emission, ammonia ( $NH_3$ ) volatilization, and nitrate ( $NO_3^-$ ) leaching (Huang et al., 2016; Said-Pullicino et al., 2014). Nitrous oxide is an important greenhouse gas (GHG) and is currently the third largest contributor to global warming, after carbon dioxide ( $CO_2$ ) and methane ( $CH_4$ ) (IPCC, 2021). It has a 298 times greater global warming potential (GWP) than  $CO_2$  and can remain in the atmosphere for an average of 114 years (IPCC, 2021). In addition to its GWP, it can cause damage to the ozone ( $O_3$ ) layer in the stratosphere, which is irreversible in the short term. Intensively managed agricultural soils emit approx. 3.5 Mt  $N_2O$ -N year<sup>-1</sup> (Pachauri et al., 2014), globally contributing almost 60% to the anthropogenic  $N_2O$  emissions and 21% to the overall  $N_2O$  emissions (IPCC, 2021). Furthermore, to date, due to agricultural intensification, atmospheric  $N_2O$  concentration has increased to 331.1 ppbv in 2018, which is about 56-61 ppbv higher than the pre-industrial level (WMO, 2020). Moreover,  $N_2O$  emissions may continue to increase under the current agricultural management strategy.

Besides  $N_2O$  emission,  $NO_3^-$  leaching caused by excessive fertilization, combined with irrigation or high precipitation, is another important way to reduce N use efficiency (Liu et al., 2019). Furthermore,  $NO_3^-$  leaching can cause environmental pollution, such as the eutrophication of groundwater, and the death of aquatic animals which threatens human health, reduces economic benefits, and limits sustainable agricultural development (Choudhury and

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Kennedy, 2005).

Quantitatively, the amount of soil N loss due to  $\text{NH}_3$  volatilization is even more important than  $\text{N}_2\text{O}$  emission (Ma et al., 2021). Excessive N fertilization and low utilization rate of plants lead to substantial  $\text{NH}_3$  volatilization in farmland, with N losses due to  $\text{NH}_3$  volatilization ranging from 1% to 47% of total nitrogen (TN) applied in farmland (Tian et al., 1998). As a result, decreasing  $\text{NH}_3$  volatilization during the process of urea hydrolysis has great potential to improve fertilizer N use efficiency.

Therefore, finding strategies to improve soil N retention is critical to achieving sustainable intensification of agricultural production. Hence, there is an urgent demand to develop a scientific approach to maintain the soil N supply at a sufficiently high level and at the same time to reduce environmental pollution caused by  $\text{N}_2\text{O}$  emissions or  $\text{NO}_3^-$  leaching.

## **1.2 Rationale and state of the art**

### **1.2.1 Biological process of $\text{N}_2\text{O}$ losses**

On a 100-year timescale,  $\text{N}_2\text{O}$  has an about 300 times higher global warming potential than  $\text{CO}_2$  and is important for stratospheric ozone depletion (Griffis et al., 2017). Therefore, reducing  $\text{N}_2\text{O}$  emissions from agricultural soil can improve soil quality, crop yield, and simultaneously have profound implications for mitigating global warming. To date, a substantial number of studies have been conducted, while the detailed  $\text{N}_2\text{O}$  production and consumption processes are not fully understood. Nitrification and denitrification, two microbiological processes, are currently considered the primary sources of  $\text{N}_2\text{O}$  emissions from agricultural soils compared to chemical processes (Chapuis-Lardy et al., 2007). For example, based on the “hole-in-the-hole” model, Firestone and Davidson (1989) concluded that nitrification (i.e., during  $\text{NH}_3$  oxidation to  $\text{NO}_3^-$ ) and denitrification (i.e., during  $\text{NO}_3^-$  reduction to  $\text{N}_2$ ) are two critical pathways of  $\text{N}_2\text{O}$  emission. In addition, nitrifier denitrification is also an important contributor to soil  $\text{N}_2\text{O}$  emission (Wrage et al., 2001).

Autotrophic nitrification is a microbial process in which  $\text{NH}_4^+$  is sequentially oxidized to nitrite ( $\text{NO}_2^-$ ) and  $\text{NO}_3^-$  under the action of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB) with  $\text{CO}_2$  as the carbon (C) source. Compared with AOB, AOA are widely distributed in terrestrial ecosystems, such as agricultural dryland soil (Leininger et al., 2006), paddy field soil (Chen et al., 2008), grassland soil (Di et al., 2010), and forest soil (Boyle-Yarwood et al., 2008). Although AOA exceed AOB in terms of quantity in many soils, the  $\text{NH}_3$  oxidation process is usually dominated by AOB (Jia and Conrad, 2009). In addition, AOB mainly exists in surface soil, hence the nitrification rate of the surface soil is usually significantly higher than that of the deep soil (Di et al., 2010).

The oxidation of  $\text{NH}_3$  to  $\text{NO}_2^-$  is the first and rate-limiting step in nitrification (Katipoglu-Yazan

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et al., 2012). However, due to the rapid conversion of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  by the action of nitrite oxidoreductase and the important role of  $\text{NO}_2^-$  in  $\text{N}_2\text{O}$  emission, the dynamics of  $\text{NO}_2^-$  in soils have constantly gained attention (Homyak et al., 2017; Venterea, 2007). Furthermore, it was also reported that  $\text{NO}_2^-$  mainly accumulated in drying soils than in wet soils (Gelfand and Yakir, 2008). In contrast to autotrophic nitrification, microbial heterotrophic nitrification requires the use of organic carbon (OC) as the primary material to build up their biomass (Song et al., 2021). Heterotrophic nitrification has a greater potential to produce  $\text{N}_2\text{O}$  emission than autotrophic nitrification in soils with low pH (especially ca. 4 to 5) (Stroo et al., 1986), but generally  $\text{N}_2\text{O}$  from heterotrophic nitrification accounts for only a small fraction of total soil  $\text{N}_2\text{O}$  emissions (Wrage et al., 2001), since this process is strictly limited by specific soil conditions (Anderson et al., 1993). For example, the OC content in the soil showed a tight relationship with the activity of heterotrophic nitrifying microorganisms by controlling the input of labile C, and oxygen ( $\text{O}_2$ ) is the critical element involved in the oxidation of  $\text{NH}_3$  to  $\text{NO}_3^-$  (Wrage et al., 2001).

Nitrification is the main process that produces  $\text{N}_2\text{O}$  in well-aerated soils, while denitrification increases in intensity at insufficient  $\text{O}_2$  supply in the soil (Bollmann and Conrad, 1998). Denitrification is the microbial/chemical process of reducing  $\text{NO}_2^-$  and  $\text{NO}_3^-$  to  $\text{N}_2$  and simultaneously producing  $\text{N}_2\text{O}$  as intermediate. Compared with chemical denitrification, microbial denitrification is assumed to play the dominant role in soil denitrification (Payne et al., 1976; Cai et al., 2012). According to the types of the reduction process, microbial denitrification can be classified into three different pathways, which are bacterial denitrification, fungal denitrification, and nitrifier denitrification (Shoun et al., 1992; Wrage et al., 2001; Strohm et al., 2007). The microbial process of denitrification is simultaneously controlled by several factors, such as soil  $\text{O}_2$  status, labile C content, soil moisture, and soil type (Burford and Bremner, 1975; Loecke and Robertson, 2009). Previous research found most of the soil  $\text{N}_2\text{O}$  emitted by microbial pathways was caused by heterotrophic facultative denitrifying bacteria (*Pseudomonas*, *Alcaligenes*, *Bacillus* et al.) (Wrage et al., 2001). Besides, fungal denitrification can also substantially contribute to soil  $\text{N}_2\text{O}$  emission (Shoun et al., 1992). Compared with denitrifying bacteria, denitrifying fungi are more adaptable to higher  $\text{O}_2$  concentration, but extremely high  $\text{O}_2$  concentration will inhibit the denitrification process (Zhou et al., 2001). Although some specific fungi (such as *Fusarium oxysporum* and *Gibberella melani*) can reduce  $\text{NO}_3^-$  or  $\text{NO}_2^-$  to produce  $\text{N}_2\text{O}$ , most denitrifying fungi can only use  $\text{NO}_2^-$  as the primary substrate for reduction (Shoun et al., 1992).

Nitrifier denitrification is a special pathway of specific AOB (such as *Nitrosomonas*, *Nitrospira*) that is coupled with nitrification and denitrification (Cai et al., 2012). In this process,  $\text{NH}_3$  is oxidized to  $\text{NO}_2^-$ , followed by the reduction of  $\text{NO}_2^-$  to nitric oxide (NO),  $\text{N}_2\text{O}$ , and  $\text{N}_2$  ( $\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ) (Wrage et al., 2001). This process can account for up to 100% of the  $\text{N}_2\text{O}$  emission from  $\text{NH}_4^+$  in soils, and it can be more relevant than classical

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denitrification, especially in an environment with conditions fluctuating between aerobic and anaerobic (Wrage et al., 2018). Chemodenitrification describes the process of chemical decomposition of  $\text{NO}_2^-$ , which is accompanied by the emission of  $\text{N}_2$ ,  $\text{N}_2\text{O}$ , and  $\text{NO}_x$  (the sum of  $\text{NO}$  and  $\text{NO}_2$ ). However, chemodenitrification only occurs at low pH conditions, implying that chemodenitrification can be the dominating process of  $\text{NO}$  and  $\text{N}_2\text{O}$  formation in strongly acidic soils, whereas in weakly acidic, neutral or alkaline soils the amount of  $\text{N}_2\text{O}$  emission from chemodenitrification was found to be significantly lower than that from microbial nitrification or denitrification (Bremner et al., 1997).

### **1.2.2 The main controls of $\text{N}_2\text{O}$ emission**

$\text{N}_2\text{O}$  emission is controlled by environmental factors such as soil  $\text{O}_2$  status, soil texture, pH, climatic conditions, as well as the available N content (Ludwig et al., 2001). Temperature and soil moisture are considered the most critical environmental factors affecting nitrification and denitrification (Davidson and Swanke, 1986), but the optimal soil moisture content is different for nitrification and denitrification (Hwang et al., 2000). For example, the highest  $\text{N}_2\text{O}$  production rate by nitrifiers was observed at around 50-60% water-filled pore space (WFPS) when the soil became partly anaerobic, but at 65-85% WFPS there were large gaseous losses from denitrification than nitrification (Davidson et al., 2000).

Soil moisture is related to the availability of  $\text{O}_2$  content and the diffusion of soil nutrients, and regulates microbial activity significantly (Parthasarathi, 2007). For example, low water availability inhibits microbial activity by reducing intracellular water potential and, thus, enzyme hydration and activity (Stark and Firestone, 1995). The moisture distribution in the soil pore space is an indicator of the availability of  $\text{O}_2$ , which controls the activity of soil microorganisms. Nitrifiers can use  $\text{O}_2$  to oxidize  $\text{NH}_3$  to  $\text{NO}_2^-$  and then oxidize  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (Wrage et al., 2001). Therefore, as the byproduct of nitrification, soils at oxic condition have a large potential for the emission of  $\text{N}_2\text{O}$  from nitrification. Conversely, soils with high water content usually have lower  $\text{O}_2$  availability in the pore space (Bollmann and Conrad, 1998; Davidson, 1993). Hence, the precipitation-induced anaerobic condition can simultaneously decrease nitrification but stimulate denitrification and the activity of other heterotrophic anaerobic microbes. In addition, Butterbach-Bahl et al. (2013) reported that  $\text{N}_2\text{O}$  emission has its optimum in the range of 70-80% WFPS depending on the soil type, while the complete denitrification with  $\text{N}_2$  as the final product will occur at even higher soil moisture levels, implying that the soil moisture level is critically affecting  $\text{N}_2\text{O}$  production, which leads to environmental pollution.

Besides soil moisture, temperature is another critical factor controlling the emission of  $\text{N}_2\text{O}$  (Schindlbacher et al., 2004). A previous study reported that warming-induced higher extracellular enzyme activity could provide soil microbes with additional sources for building



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up microbial biomass (Bell et al., 2010). Simultaneously, the release of N through the decomposition of soil organic matter (SOM) could supply the substrate for nitrification and/or denitrification (Chen et al., 2013). In the presence of sufficient O<sub>2</sub>, autotrophic nitrifiers can use the oxidation of NH<sub>4</sub><sup>+</sup> as energy source to fix CO<sub>2</sub> for the buildup of their biomass and produce the N<sub>2</sub>O as a by-product of the nitrification process (Kool et al., 2010). On the other hand, climate warming will lead to an increase in soil microbial metabolism and respiration, which is eventually accompanied by an increase in soil O<sub>2</sub> consumption and intensified anaerobic state in the soil without moisture limitation (Liu et al., 2017). As the critical enzymes in the denitrification process, nitrate reductase and nitrite reductase activities increase simultaneously (Fowdar et al., 2022). Hence, the production of N<sub>2</sub>O, which is the intermediate product of the denitrification process, might increase if the last step of denitrification (N<sub>2</sub>O to N<sub>2</sub>) is limited. Therefore, the emission of N<sub>2</sub>O, an intermediate product of the denitrification process, could increase during the process.

It is well recognized that, besides available N content, the N<sub>2</sub>O emission is controlled by the labile C content of the soil (Koskinen and Keeney, 1982; Parkin, 1987). Under anaerobic conditions, heterotrophic denitrifiers use oxidized N as terminal electron acceptor and OC as the electron donor and use the electron transfer to perform oxidative phosphorylation (Wrage et al., 2001). The release of labile C from SOM decomposition can provide energy for microbial activity and the basic materials for the development of the microbial community in C-limited systems, leading to more N<sub>2</sub>O emissions through denitrification if the soil O<sub>2</sub> status is low enough (Brackin et al., 2014).

Soil pH is a critical factor affecting N<sub>2</sub>O emissions. Previous studies with pure cultures of denitrifiers grown in the laboratory found a strong effect of pH on denitrification with maximal rates at near-neutral pH (Valera and Alexander, 1961; Thomas et al. 1994; Thomsen et al. 1994). However, some studies found that the relative contribution of N<sub>2</sub>O production of denitrification increases at lower pH (Thomsen et al., 1994; Van den Heuvel et al., 2010). For example, Cheng et al. (2015) reported that denitrification dominated N<sub>2</sub>O production below a pH threshold of approximately 4.4. Another study found that denitrification will be the dominant source of N<sub>2</sub>O production in acidic soils (Van den Heuvel et al., 2010).

Soil texture is an essential factor determining the size and distribution of soil pores and, hence, affecting soil aeration and O<sub>2</sub> availability, and ultimately the relative contributions of nitrification and denitrification to total N<sub>2</sub>O flux (Li et al., 2021b; Bateman and Baggs, 2005; Maag and Vinter, 1996; Khalil et al., 2004). Soils with finer textures and, thus, smaller particle size characteristics usually have higher moisture content at the same matrix potential, which easily leads to the generation of anoxic microhabitats, which is favorable for the denitrification process (Gaillard et al., 2016). Fine-textured soils usually have higher total N<sub>2</sub>O emissions than coarse-textured soils (Van Groenigen et al., 2004). For example, Li et al. (2021a) found that the

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N<sub>2</sub>O emission rate from silty soil is significantly higher than that in sandy soil. The reason is that sandy soils with larger pores provide microorganisms with higher O<sub>2</sub> content, providing better conditions for nitrification compared to clay soils. Conversely, anaerobic conditions are more common in dense, heavy clay soils, characterized by much smaller pore size distribution (Bhattarai et al., 2006). Furthermore, the anaerobic conditions often found in clay soils create a suitable environment for N<sub>2</sub>O emission through denitrification.

Phosphorus (P) is a crucial component of deoxyribonucleic acid (DNA), phospholipids, adenosine triphosphate (ATP), and other essential molecules in all living organisms. The availability of P in soil affects soil biogeochemical processes, together with the development of microbial biomass and community composition and the decomposition of high carbon amendments (HCA), and is thus pivotal in supporting crop productivity (Brookes et al., 1984). Previous research reported that, although the soil is characterized by high biological diversity, structural complexity, and spatial heterogeneity, there is a remarkably consistent C:N:P ratio in both total soil pools and the soil microbial biomass. The average C:N:P ratio was reported to be 186:13:1 in the soil and 60:7:1 in microbial biomass at the global scale (Cleveland and Liptzin, 2007). As a result, the availability of P in the soil can be a limiting factor for the decomposition of HCA, as well as the subsequent development of the soil microbial community stimulated by C input to the soil.

### **1.2.3 Direct and indirect effects of organic soil amendments on soil N retention and N losses**

Many studies have been conducted on increasing soil N retention and reducing N losses by different methods, for instance, by application of nitrification inhibitors to suppress the bacterial oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> in the soil by AOB, such as *Nitrosomonas* (Zerulla et al., 2001), or by application of biochar to increase soil water holding capacity (WHC) and NH<sub>4</sub><sup>+</sup> adsorption (Liu et al., 2017). Besides, applying HCA to achieve N retention has also received widespread attention (Curtin et al., 1998; Reichel et al., 2018; Wu et al., 2013).

If C resources are abundant, the conversion of inorganic N in the soil to organic N through the formation of microbial biomass is an alternative method to reduce soil N losses (Reichel et al., 2018). Cleveland and Liptzin (2007) suggested that despite the high biodiversity, structural complexity, and spatial heterogeneity of soils, the C:N:P ratio of soil microbial biomass is remarkably constant on a global scale. Types of HCA with C:N ratios above 60 contain large amounts of microbially decomposable cellulose and hemicellulose, which could be a potential C source for the microorganisms hence affecting microbial biomass growth (Reichel et al., 2018). For example, during decomposition of HCA, microorganisms will be forced to take up N from the soil to maintain their narrow C:N ratio of approximately 7 (Cheshire et al., 1999).

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In addition, the effect of HCA on N retention is determined by their biodegradability, e.g., different decomposition rates, C:N ratios, and initial nutrient contents. Wheat straw (C:N ratio of 50-100), for instance, contains a large fraction of easily decomposable organic C, which caused a rapid N retention in the microbial biomass of up to 42 kg N ha<sup>-1</sup> when applied at a rate of 4.5 t C ha<sup>-1</sup>, with the caveat that under the optimal microbial growth conditions in the laboratory the N retention lasted only several weeks (Reichel et al., 2018). Other HCA, such as leonardite and sawdust as byproducts of lignite mining and wood processing, respectively, have promising properties for increasing the C and N storage in the soil over a longer period. Leonardite (*i.e.*, an oxidized form of lignite) is characterized by similar C:N ratios as wheat straw but, in addition, contains humic acids and a smaller fraction of easily available C (Fowkes and Frost, 1960). Spruce sawdust with its very large C:N ratio of about 400 has a large fraction of C bound in lignin, which makes it less decomposable for bacteria, but potentially favors fungal growth and N immobilization in the long term (Reichel et al., 2018; Vano et al., 2011). However, sawdust is widely used as a renewable fuel source and for producing wood particle boards, and leonardite as a fossil fuel is not a renewable resource, suggesting that it should not be recommended for widespread application in agriculture. Still, they are excellent model substances to research into how the properties of HCA affect soil N retention, finally opening avenues to new, engineered organic soil amendments based on renewable resources.

It was reported that the addition of HCA also had a profound effect on soil physical properties, e.g., WHC, bulk density (BD), aggregate sizes, etc. (Barzegar et al., 2002; Blanco-Canqui et al., 2007; Kumari et al., 2018). Kumari et al. (2018) showed that incorporating HCA into soil, especially with high osmotic pressure, can increase the soil WHC significantly, enhancing the great potential to decrease NO<sub>3</sub><sup>-</sup> leaching. Moreover, HCAs are reported to reduce the BD and aggregate sizes, which is closely related to the availability of soil O<sub>2</sub> (Chaudhari et al., 2013; Arvidsson, 1998; Hevia et al., 2003). Previous research showed that the activity of soil microorganisms is enhanced under aerobic conditions, which increases the immobilization of mineral N (Schmidt et al., 1999). Oppositely, another study found that elevated temperature shifts N cycling from microbial immobilization to enhanced mineralization (Dai et al., 2020). Consequently, due to the complex response mechanisms to HCA, the processes of N loss from the soil to the atmosphere and groundwater should be more comprehensively assessed.

Besides NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, as the substrate of nitrification, also determines the intensity of soil N leaching, nitrification, and denitrification processes. Therefore, physical adsorption of NH<sub>4</sub><sup>+</sup> after HCA addition is another efficient way to reduce N losses (Cong et al., 2017). However, this process is limited by the relationship between the competing ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) and NH<sub>4</sub><sup>+</sup>. The adsorption capacity of NH<sub>4</sub><sup>+</sup> in solutions containing monovalent cations (Na<sup>+</sup> and K<sup>+</sup>) is higher than that of solutions containing divalent cations (Ca<sup>2+</sup> and Mg<sup>2+</sup>) because

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divalent cations are stronger competitors for adsorption sites than monovalent cations, as divalent cations occupy twice as much adsorption sites due to their divalent charge (Yang et al., 2018a).

Soil C can provide the substances necessary for the development of microbial communities. In general, soil microorganisms will immobilize N from the soil to maintain the internal C:N balance (i.e., they maintain a strict homeostasis), especially after adding substrates with high C concentration. Previous studies have shown that the application of HCA can improve N retention in agroecosystems to some extent (Li et al., 2021b; Yang et al., 2015). However, this positive effect generally occurs much later, albeit for a longer duration, due to the limited substrate decomposition rates. Conversely, the development of microbial communities would significantly and quickly increase after the addition of labile C substrates such as glucose, while this positive effect will disappear once the substrate is consumed (Blagodatskaya et al., 2009; Xu et al., 2020). Hence, the properties of C in different substrates could affect the N retention significantly.

#### **1.2.4 Effect of organic soil amendments on crop yield**

Crop production and yield is limited by various factors, such as temperature, precipitation, and soil nutrient availability (Challinor et al., 2014; Hepperly et al., 2009). The application of HCA is widely believed to maintain soil fertility on the basis of biological and physical processes (Li et al., 2021a; Reeves et al., 1997). For instance, 1) the biological process of microbial N retention by N assimilation (i.e., the buildup of microbial biomass), and 2) the physical process of increasing the WHC of the soil and, thus the volume of leachate. At the same time, HCA will intensify the competition of microbes and plants for N (Singh et al., 2007). However, the effect of HCA on soil N retention is limited by factors such as the decomposability and C:N ratio. Thus, to date, several studies on the effect of HCA on crop yield have been conducted, but the effects of soil amendments on crop yield still need to be fully understood. For example, Akhtar et al. (2018) showed that returning wheat straw to the field at a rate of  $5.0 \times 10^3 \text{ kg ha}^{-1}$  increased maize crop productivity by 7%. However, the statistical analysis by Xie et al. (2017) showed that maize straw return at a rate of  $5.0 \times 10^3 \text{ kg ha}^{-1}$  reduced wheat yield by 5.1%. Organic soil amendments increased soil WHC and reduced  $\text{NO}_3^-$  leaching while providing sufficient water to plant roots (Goel et al., 2019). Besides, HCA can also increase the  $\text{O}_2$  content in the soil, stimulate microbial activity, and accelerate the decomposition of SOM, thereby providing extra N resources for plant growth and yield (Bedada et al., 2014). Conversely, Islam et al. (2022) found that, without fertilizer application, HCA return did not lead to an improvement of crop yield in a mono-cropping system (spring wheat or maize in a year) or even decreased crop yield by 2.4% in a double-cropping system (winter wheat-summer maize rotation in a year) in China. Compost (a specific HCA) is a good source of nutrients while increasing SOM content with

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positive effects on soil structure, WHC, nutrient retention (Hargreaves et al., 2008; Mohanty et al., 2013), and crop productivity (Erhart et al., 2005; Tejada and Gonzalez, 2003). In addition, compost application suppresses many diseases caused by soil-borne plant pathogens (Cotxarrera et al., 2002). However, improper use of compost may cause severe environmental pollution. For example, various gases released during composting, especially  $\text{NH}_3$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ , can affect air quality; moreover, water quality can be affected by mineral N loss, i.e.,  $\text{NO}_3^-$  leaching (Peigné and Girardin, 2004). Therefore, the positive impact of compost application on sustainable agricultural development may be offset by inappropriate application.

### **1.2.5 N in the soil after crop harvest**

Whether crop residues are returned to the field after harvest or not is critical to controlling N emissions and leaching (Li et al., 2021b). Nitrogen retention is of particular importance when residues of N-rich crops, such as field bean (*Vicia faba* L.), oilseed rape (*Brassica napus* L.), sugar beet (*Beta vulgaris* L.), and potato (*Solanum tuberosum* L.), are mineralized in soil. Nutrient-rich residues can release around 20-60 kg N ha<sup>-1</sup> of mineral N, available for the microbial community, for plant uptake (if present), or for gaseous and leaching losses, depending on the quantity and quality of the residues (Döhler, 2009). Due to a mismatch between N mineralization of crop residues and microbial/plant N uptake during the period between harvest and crop seeding, as well as during the period of slow plant growth in winter, agricultural soils bear a high risk of  $\text{N}_2\text{O}$  emission or  $\text{NO}_3^-$  leaching (Chaney, 1990). Crops, such as winter wheat or winter barley, generally grow slowly during winter due to physiological constraints (Sieling et al., 1999). Thus, the N released by mineralization of plant and animal debris in excess of plant requirements is the primary source of nutrient loss in the winter (Matzner and Borken, 2008). Balancing soil N supply and plant demand by capturing excess N in times of N excess and resupply to the crops in times of high plant N demand therefore holds great potential for environmentally friendly and sustainable agriculture.

### **1.2.6 Lysimeter-an advanced tool for the measurement of N cycling**

For the study of the interaction between soil nutrients and crop yield, traditional field trials are the most commonly used method. However, it is difficult to monitor changes in nutrient losses due to leaching and runoff in the field in real-time on a long-term and frequent basis (Pütz et al., 2018). Laboratory incubation experiments are another research method in addition to field experiments under more controlled conditions. However, the sample size for such incubation experiments is usually small, and the conditions may be different from the natural environment (Leng et al., 2019). Lysimeters are an ideal system for quantifying water, C and N balances and losses in leachate and gaseous forms. In addition, outdoor lysimeters provide an intermediate link between laboratory and field conditions, combining their advantages and avoiding

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shortcomings. As such, it is a valuable experimental tool to study soil biogeochemical processes under controlled but realistic conditions, and to measure complex interactions between soils, plants, water, nutrients, and chemicals (Kay et al., 2004; Pütz et al., 2018).

### **1.3 Objectives and outline of the thesis**

The objective of this thesis was to explore the potential relationship between HCA application and N availability in the soil, intending to find ways to optimize nutrient use efficiency while maintaining or even improving crop yield and quality. The main questions that this thesis aimed to resolve were the following:

- (1) Which of the selected HCA types (wheat straw, sawdust, or leonardite) will be the most effective to retain N in agricultural soil?
- (2) Can microbial N retention be increased by P co-application?
- (3) Can the addition of exogenous labile C improve the N retention potential of more recalcitrant HCA?
- (4) Which effect does compost addition have on the relationship of crop yield and N loss?

This thesis contains the results of a meta-analysis, two incubation experiments, and a lysimeter experiment. The meta-analysis was conducted to investigate the effect of the return of different crop residue types to the field on soil N<sub>2</sub>O emissions and NO<sub>3</sub><sup>-</sup> leaching for various soil conditions and land management practices and showed that crop residues can increase N<sub>2</sub>O emission but mitigate the NO<sub>3</sub><sup>-</sup> leaching. In the first incubation experiment, wheat straw, sawdust and leonardite, which have different C:N ratios, decomposition rates and labile C contents, were selected to study the effect of labile C on GHG emissions and soil N availability at different P levels and soil conditions. The hypotheses of this experiment were that 1) wheat straw is most effective in retaining N in agricultural soils because it introduces a large amount of labile C into the soil during its decomposition by soil microorganisms, and 2) P availability in soil affects soil biogeochemical processes as well as microbial biomass development. Hence, P application can increase N retention, especially in soil with high nutrient content.

The second incubation experiment was based on the first incubation experiment, with the addition of <sup>13</sup>C-labeled glucose as an additional C source, thus investigating whether adding a labile C source would accelerate the decomposition process of a less decomposable, exogenous C source, thereby increasing the N retention capacity. We hypothesized that 1) simultaneous application of glucose and P fertilizer would improve the N retention potential of recalcitrant HCA, such as sawdust, through stimulating HCA decomposition (i.e., priming), reflected in increased CO<sub>2</sub> emission, and 2) HCA application would reduce N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> loss due to enhanced microbial immobilization of mineral N in the soil. The lysimeter experiment investigated the effect of using green-cutting compost as a C additive on soil N retention and crop yield under two different P supply conditions. Four hypotheses guided this experiment: 1)

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compost application will not lead to a significant increase in GHG emissions due to efficient (re)cycling of C, N, and P in the soil-plant system; 2) additional mineral P fertilization stimulates substrate mineralization and thus releases additional N, which further increases plant growth and yield; 3) combined application of compost and mineral N and P fertilizer will not lead to increased N and P leaching due to efficient microbial and plant immobilization of nutrients; 4) increasing compost application rates will enhance plant growth and yield compared to the control without compost due to enhanced supply of nutrients and water to the plants.

In summary, in this thesis, the study on the effect of HCA application on soil N retention mainly focused on soil N<sub>2</sub>O emission, NO<sub>3</sub><sup>-</sup> leaching, and crop yield under different C and P levels. This thesis presents theoretical support for sustainable agriculture, which aims to optimize nutrient efficiency and reconcile yield production and environmental protection.

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## Chapter 2

### **Return of crop residues to arable land stimulates N<sub>2</sub>O emission but mitigates NO<sub>3</sub><sup>-</sup> leaching: a meta-analysis**

Based on:

Li Z, Reichel R, Xu Z, Vereecken H, Brüggemann N. (2021) Return of crop residues to arable land stimulates N<sub>2</sub>O emission but mitigates NO<sub>3</sub><sup>-</sup> leaching: a meta-analysis. *Agron. Sustain. Dev.* 41, 1-17. <https://doi.org/10.1007/s13593-021-00715-x>



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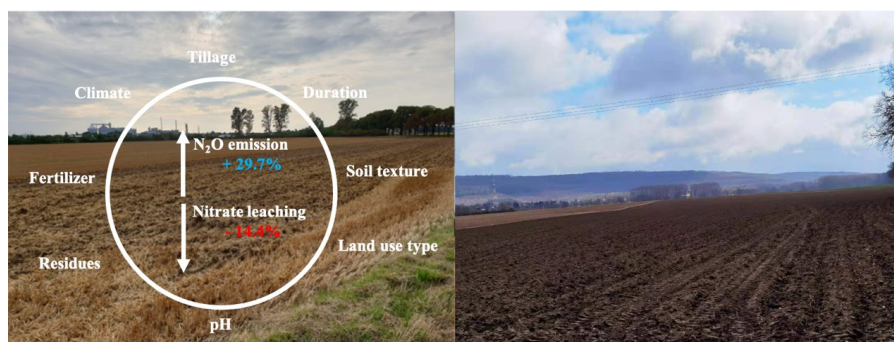
## 2.1 Introduction

Nitrous oxide emission and  $\text{NO}_3^-$  leaching from intensively managed cropland cause significant threats to adjacent environmental compartments (Bodirsky et al., 2012; Yang et al., 2018b). Nitrous oxide has 298 times greater GWP than  $\text{CO}_2$  and accelerates  $\text{O}_3$  depletion (Ravishankara et al., 2009). Intensively managed agricultural soils emit approx.  $3.5 \text{ Mt N}_2\text{O-N yr}^{-1}$  (Pachauri et al., 2014), globally contributing almost 60% to the anthropogenic  $\text{N}_2\text{O}$  and 21% to the overall  $\text{N}_2\text{O}$  emission (IPCC, 2021). Nitrate leaching is another critical N loss pathway that leads to surface water eutrophication as well as groundwater pollution (Di and Cameron, 2002). Therefore, finding strategies for improving N retention in soil is highly relevant for making intensive agricultural production more sustainable (Fig 2.1).

Return of crop residues with high C content has great potential to improve N retention in soils (Yang et al., 2015). The annual production of crop residues reached nearly 4 billion metric tons at the beginning of the 21<sup>st</sup> century (Lal, 2005). This indicates that appropriate utilization of crop residues with high N retention capacity could maintain soil fertility and reduce N losses effectively (Liu et al., 2014; Powlson et al., 2008). The C:N ratio of plant tissue is an important indicator of residue quality and decomposability, which is closely related to the immobilization of N, mainly by stimulating N retention in microbial biomass and increasing N sorption by the humus fraction (Chen et al., 2013). Crop residues with a low C:N ratio ( $< 25$ ), such as legume residues, can be easily decomposed by the soil microbial community in a short time period, resulting in the release of available N, which can further undergo soil nitrification and denitrification (Reichel et al., 2018). The release of available N from crop residues can be beneficial for increasing crop yield in the next growing season, but only if it is not lost from the soil beforehand (Mooshammer et al., 2014; Whitmore and Groot, 1997). However, residue decomposition can create anaerobic hotspots in the soil, which may stimulate denitrification, hence partially thwarting the benefit of soil C sequestration (Zhou et al., 2017b). Crop residues with C:N ratio greater than 25 are usually more recalcitrant and force microorganisms to take up N from soil to meet their N need, i.e. the decomposition of crop residues with high C:N ratio causes subsequent microbial N immobilization. As a consequence, the temporary shortage of soil N might restrict nitrification and denitrification, with beneficial effects on  $\text{NO}_3^-$  and  $\text{N}_2\text{O}$  losses (Aulakh et al., 2001; Cleveland and Liptzin, 2007).

Cropland management strategies can affect the impact of residues return on soil N retention (Xia et al., 2014). For example, the application rate and composition of synthetic fertilizers affect soil nutrient availability, and different plowing methods can strongly affect the soil aggregate structure (Van Kessel et al., 2013; Xia et al., 2018). The effect of crop residue return on soil N retention is also influenced by soil properties. For instance, soil pH regulates the decomposition rate of crop residues providing N to nitrifiers and denitrifiers (Chen et al., 2013).

Soil pH values of 7 or higher are favorable for denitrification (Wijler and Delwiche, 1954), and the influence of crop residue return on the reduction of soil  $\text{N}_2\text{O}$  emissions was found to be most significant at pH 7.1-7.8 (Chen et al., 2013). Soil physical properties like pore size distribution, BD, and WHC content are key variables that control crop residue degradation and N transformation in soil (Chen et al., 2013). Climatic conditions, such as mean annual temperature (MAT) and precipitation (MAP), can also affect  $\text{N}_2\text{O}$  emissions and  $\text{NO}_3^-$  leaching in combination with crop residue application (Butterbach-Bahl et al., 2013; Liu et al., 2017).



**Figure 2.1** Example of crop residue management after harvest, which affects soil N retention ( $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching). Left: Crop residue incorporation, right: crop residue removal. Photographs: Zhijie Li.

Even though several meta-analyses evaluating the responses of N losses to residue return have been published, to our knowledge a comprehensive assessment accompanied by cropland management strategies on soil N retention,  $\text{N}_2\text{O}$  emission, and  $\text{NO}_3^-$  leaching is lacking so far. Therefore, we conducted a global meta-analysis including 345 observations from 90 studies to systematically evaluate the overall effect of crop residue return on soil N retention and N losses ( $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching) (Fig. S2.1). We hypothesize that 1) residue return will stimulate  $\text{N}_2\text{O}$  emission, but mitigate  $\text{NO}_3^-$  leaching; 2) the effectiveness of residue return on N losses will be governed by soil type, crop residue characteristics, climatic conditions, and cropland management.

## 2.2 Materials and methods

### 2.2.1 Data sources

To find the relevant literature for our meta-analysis, we used Web of Science, Google Scholar, and China National Knowledge to search for publications focusing on the comprehensive analysis of residue return and its effect on soil N losses, published before 11 January 2020. The

search terms were ‘(residue OR straw OR OA) AND (N<sub>2</sub>O emission OR NO<sub>3</sub><sup>-</sup> leaching)’. In addition, we limited our selection to those publications of experimental studies that fulfilled the following criteria: (a) the study was based on practice-relevant field, mesocosm, and lysimeter experiments, excluding lab experiments; (b) N losses (N<sub>2</sub>O emission or NO<sub>3</sub><sup>-</sup> leaching) in the experiment were measured for at least one growing season (observations made over several growing seasons were averaged); (c) experimental and control plots had been established in the same ecosystem and included at least one comparison of N losses; (d) statistical information such as mean values of N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> leaching, standard deviation (SD), and samples size in the experiment were directly extractable from the tables of the published articles or were extracted from the graphs with the GetData Graph Digitizer software (version 2.26: <http://getdata-graph-digitizer.com/download.php>).

The selected studies provided information on (i) geographic coordinates (latitude and longitude), (ii) climatic zones ((sub)tropical, and temperate), (iii) land use type (paddy soil and upland soil), (iv) MAT and MAP; (v) soil texture, soil organic carbon (SOC), TN, extractable P (EP), C:N ratio, pH, and BD; (vi) fertilizer composition (single N fertilizer or NPK compound fertilizer), N fertilizer types (urea, NH<sub>4</sub>NO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, or NH<sub>4</sub>HCO<sub>3</sub>), and application times (number of fertilizer applications per growing season), (vii) residues type, tillage method, and experimental duration. Crop residues were divided into low C:N residues with C:N < 25, and in high C:N residues with C:N ≥ 25.

As some studies did not include the information on climate or soil properties, we obtained the missing data from the World Climate Database (<https://www.worldclim.org>) and the Harmonized World Soil Database v1.2 (FAO, 2012) according to the geographic locations. The resolution of the data was 30 seconds for the World Climate Database and 5 minutes Harmonized World Soil Database. If the geographic locations were given in the unit of decimal degrees (DD), we converted them to degrees/minutes/seconds (DMS) with a DMS-DD converter (<https://www.fcc.gov/media/radio/dms-decimal>). Based on these selection criteria, we identified 345 observations from 90 peer-reviewed articles on a global scale. Specifically, the number of observations for N<sub>2</sub>O emission was 255 (Table S2.2), and 90 for NO<sub>3</sub><sup>-</sup> leaching (Table S2.3).

### 2.2.2 Data analysis

The effect size, evaluating the responses of N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> leaching to crop residue return, is defined as the natural logarithm of the response ratio (*lnRR*) (Hedges et al., 1999).

$$\ln RR = \ln \left( \frac{X_t}{X_c} \right) \quad (1)$$

where  $X_t$  and  $X_c$  are the mean value of the variable with (treatment) or without (control) crop residue return, respectively.

The variance ( $v$ ) of each study was estimated as:

$$v = \frac{S_t^2}{n_t X_t^2} + \frac{S_c^2}{n_c X_c^2} \quad (2)$$

where  $n_t$  and  $n_c$  are the sample sizes of each variable in treatment and control groups, while  $S_t$  and  $S_c$  are the SD for the treatment and control groups, respectively. If only the standard error (SE) was given, the corresponding SD was re-calculated.

This meta-analysis was performed using a nonparametric weighting function, and the weighting factor ( $W_{ij}$ ), weighted response  $\ln RR_{++}$ , and standard error  $S(\ln RR_{++})$  were calculated as:

$$W_{ij} = \frac{1}{v} \quad (3)$$

$$\ln RR_{++} = \frac{\sum_{i=1}^m \sum_{j=1}^k W_{ij} \ln RR_{ij}}{\sum_{i=1}^m \sum_{j=1}^k W_{ij}} \quad (4)$$

$$S(\ln RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^m \sum_{j=1}^k W_{ij}}} \quad (5)$$

where  $m$  is the number of groups and  $k$  is the number of comparisons.

The 95% bootstrap confidence interval (CI) of  $\ln RR_{++}$  was calculated according to Curtis and Wang (1998) by bootstrapping of 4999 iterations (Rosenberg et al., 1997):

$$95\%CI = \ln RR_{++} \pm S(\ln RR_{++}) \quad (6)$$

If the 95% CI of  $\ln RR_{++}$  for a given variable overlapped with zero, the response to crop residue return was considered as not significantly different between treatment and control.

The frequency distribution of  $\ln RR$ , reflecting the variability of crop residue effects among individual studies, was calculated with the following Gaussian function:

$$y = \alpha \exp \left[ -\frac{(x - \mu)^2}{2\sigma^2} \right] \quad (7)$$

where  $y$  is the frequency of  $\ln RR$  values within an interval,  $x$  is the mean value of  $\ln RR$  for that interval,  $\mu$  and  $\sigma^2$  are the mean and variance across all  $\ln RR$  values, respectively, and  $\alpha$  is a coefficient indicating the expected number of  $\ln RR$  at  $x = \mu$ .

The statistical tests were considered significant at the  $P < 0.05$  level. All of the meta-analysis procedures were conducted using MetaWin 2.1 software (Sinauer Associates, Inc., Sunderland, MA, USA), and statistical analyses were performed using SPSS 21.0 (IBM Deutschland GmbH, Ehningen, Germany) for Windows.

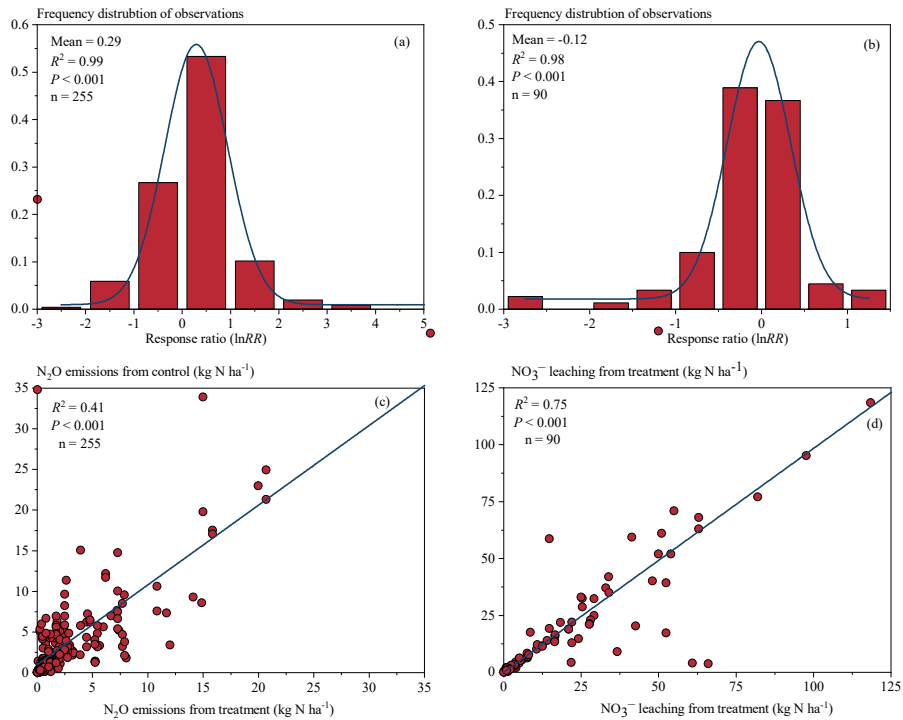
### 2.2.3 Sensitivity analysis and publication bias

We conducted a sensitivity analysis to estimate the effects of crop residue return on  $N_2O$  emissions and  $NO_3^-$  leaching. First, a mixed model was established to calculate  $\ln RR_{++}$  and reduce the disturbance of extreme variables simultaneously. Then we excluded  $\ln RR$  randomly and decreased the  $\ln RR$  numbers included in  $\ln RR_{++}$  from 100% to 60%. Once the  $\ln RR_{++}$

presented a significant difference between each other, it passes the sensitivity analysis unsuccessfully. Potential publication bias was analyzed with funnel plot analysis and Egger's indicator test (Egger et al., 1997) with the Stata Statistical Software (version 16,2019, StataCorp LLC, College Station, TX, USA), using a 95% confidence interval.

## 2.3 Results

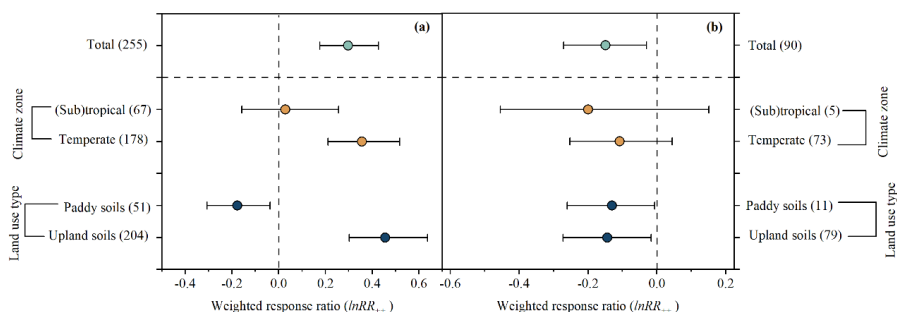
Our sensitivity analysis showed that the results of the meta-analysis did not change significantly after stepwise reduction of the number of observations, demonstrating the reliability of our analysis (Fig. S2.2). In addition, no publication bias was found when our data were analyzed with the funnel plot and Egger's test (Fig. S2.3).



**Figure 2.2** Frequency distributions of response ratios ( $\ln RR$ ) of  $N_2O$  emission (a) and  $NO_3^-$  leaching (b) to crop residue application. The curves were fitted with a Gaussian function, and the mean value, coefficient of determination ( $R^2$ ) and significance level ( $P$ ), and sample size ( $n$ ) are shown. Linear regression between  $N_2O$  emission (c) and  $NO_3^-$  leaching (d) from control and treatment.

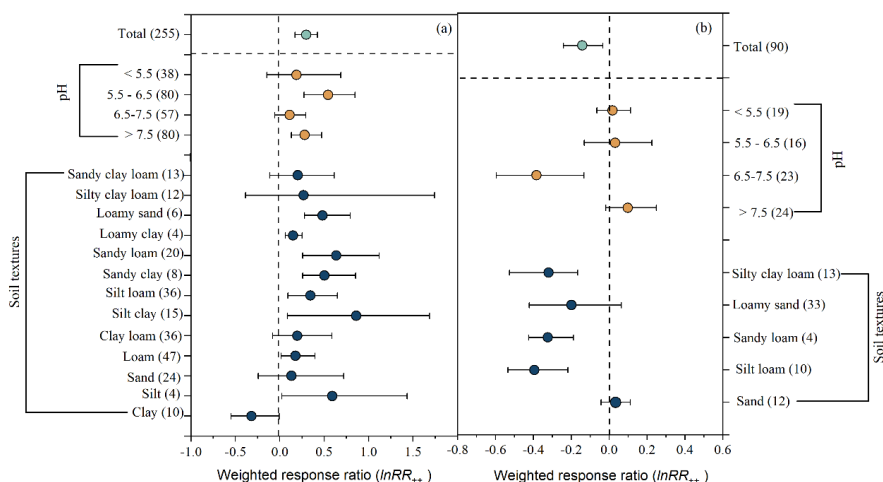
The individual  $\ln RR$  values of soil  $N_2O$  emissions or  $NO_3^-$  leaching were all normally distributed, but varied greatly among the observations (Fig. 2.2a, b). The  $\ln RR$  of  $N_2O$  emission

exhibited a great variability among the different studies, with a range from  $-2.26$  to  $3.06$  (Fig. 2.2a), while the mean value of  $\ln RR$  across all the 90 pairs of  $\text{NO}_3^-$  leaching was  $-0.12$  (range from  $-2.85$  to  $1.39$ ) (Fig. 2.2b). A higher  $\text{N}_2\text{O}$  emission, but lower  $\text{NO}_3^-$  leaching was observed from cropland soil amended with crop residues compared to the non-amended control, but the differences were not significant (Fig. 2.2c, d).



**Figure 2.3** Weighted response ratios ( $\ln RR_{++}$ ) of soil  $\text{N}_2\text{O}$  emission (a) and  $\text{NO}_3^-$  leaching (b) to crop residue application in different climate zones and land-use types. Mean effect and 95% CIs are shown. When the CI does not overlap with zero, the response is considered significant. Numbers in parentheses indicate the number of observations.

The  $\ln RR_{++}$  of soil  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching to crop residue application differed between climate zones (Fig. 2.3, Table S2.1). Overall, crop residue application significantly stimulated  $\text{N}_2\text{O}$  emission by 29.7%, with a significantly higher increase of 35.7% in the temperate zone (Fig. 2.3a, Table S2.2). In contrast, no significant effect of crop residue application on  $\text{N}_2\text{O}$  emission was observed for tropical zones (Fig. 2.3a, Table S2.2). The mean value of  $\ln RR_{++}$  across all responses of  $\text{NO}_3^-$  leaching to crop residue application was  $-0.14$  (Fig. 2.3b, Table S2.3). The response of  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching to residue application was affected by land use type. Upland soil amended with crop residues showed a significant increase of  $\text{N}_2\text{O}$  emission, which was 46% higher than control (Fig. 2.3a, Table S2.2). Conversely, it decreased the  $\text{N}_2\text{O}$  emission by 18% in paddy soil (Fig. 2.3a, Table S2.2). In contrast, crop residue application mitigated  $\text{NO}_3^-$  leaching in upland and paddy soil simultaneously (Fig. 2.3b, Table S2.3). The  $\ln RR$  of  $\text{N}_2\text{O}$  emission was significantly and positively correlated with latitude, but not with longitude, MAT, and MAP (Table 2.1). In contrast, the  $\ln RR$  of  $\text{NO}_3^-$  leaching had a significant and positive relationship with longitude, but no significant relationship with latitude, MAT, and MAP (Table 2.1).

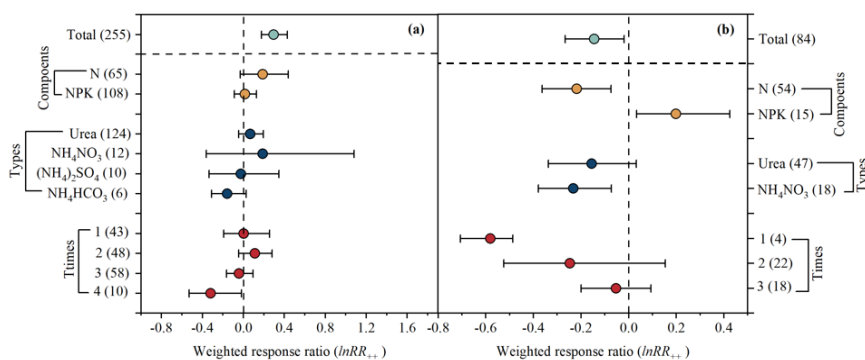


**Figure 2.4** Weighted response ratios ( $\ln RR_{++}$ ) of soil  $N_2O$  emission (a) and  $NO_3^-$  leaching (b) to crop residue application in dependence on soil pH and soil texture. The mean effect and 95% CIs are shown. When the CI does not overlap with zero, the response is considered significant. Numbers in parentheses indicate the number of observations.

Soil properties had a significant effect on the  $\ln RR_{++}$  of  $N_2O$  emission and  $NO_3^-$  leaching (Table 2.1). Compared with the control, crop residue application significantly increased  $N_2O$  emission by 54.0% when soil pH 5.5-6.5, and by 28.9% for soil pH > 7.5 (Fig. 2.4a, Table S2.2). The  $\ln RR_{++}$  of  $N_2O$  emission showed negative linear correlations with pH, SOC, TN, EP, and BD, whereas the opposite was true for the correlation with C:N (Table 2.1). Generally, crop residue application mitigated soil  $NO_3^-$  leaching, and the decrease was significant for soil pH 6.5-7.5 (Fig 2.4b). Moreover, the  $\ln RR$  of  $NO_3^-$  leaching to crop residue application was significantly correlated with SOC, TN, EP, and BD (Table 2.1). Crop residue return caused a particularly strong and significant increase in soil  $N_2O$  emissions except for soil with clay texture, indicating that clay content is an important determinant of the soil  $N_2O$  emission response to crop residue application (Fig. 2.4a). Compared with the control,  $NO_3^-$  leaching from sandy loam, silty clay loam, and silt loam showed a significant negative response to crop residue application, with a decrease of 32.4%, 32.0%, and 39.5%, respectively (Fig. 2.4b, Table S2.3).

**Table 2.1** Linear or logarithmic regression analysis between the  $\ln RR$  of  $N_2O$  emission and  $NO_3^-$  leaching to residue application as a function of latitude; longitude; MAT: mean annual temperature; MAP: mean annual precipitation; pH; SOC: soil organic carbon; TN: total nitrogen; C:N; EP: extractable phosphorus; BD: bulk density. n: number of observations included in the correlation analysis; R: Pearson's correlation coefficient; *P*: *P*-value of correlation analysis and the values in bold indicate statistical significance at  $P < 0.05$  probability level.  $\ln RR$ : natural logarithm of the response ratio. Logarithmic regression analysis was chosen when the result of linear regression analysis was insignificant. An asterisk (\*) indicates a logarithmic regression relationship between variables and  $\ln RR$  of  $N_2O$  emission and  $NO_3^-$  leaching.

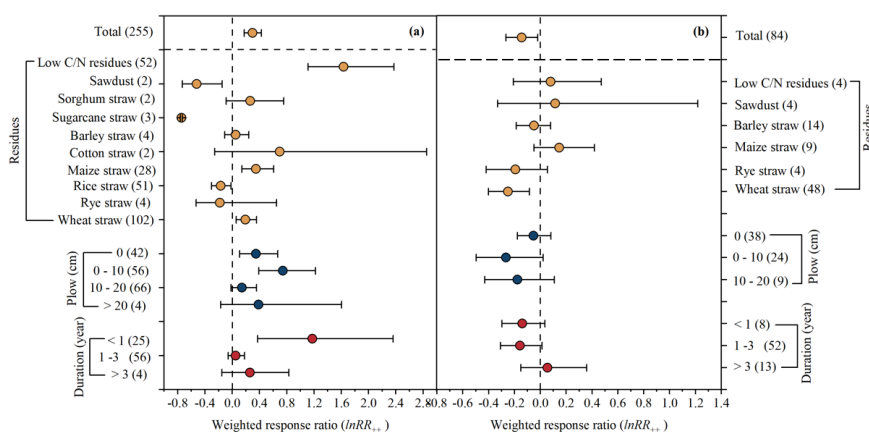
Variables	$N_2O$ emission $\ln RR$			$NO_3^-$ leaching $\ln RR$		
	<i>n</i>	<i>R</i>	<i>P</i>	<i>n</i>	<i>R</i>	<i>P</i>
Latitude	245	0.142	<b>&lt;0.01</b>	77	-0.191	>0.05
Longitude	245	-0.194	<b>&lt;0.05</b>	77	0.248	<b>&lt;0.05</b>
MAT	245	-0.244	<b>&lt;0.001</b>	77	0.088	>0.05
MAP	245	-0.296	<b>&lt;0.001</b>	77	0.057	>0.05
pH	255	-0.047	>0.05	82	-0.023	>0.05
SOC	241	-0.041	>0.05	76	-0.156	<b>&lt;0.05*</b>
TN	209	-0.132	>0.05	45	-0.347	<b>&lt;0.05</b>
C:N	209	0.135	<b>&lt;0.01*</b>	44	-0.270	>0.05
EP	76	-0.368	<b>&lt;0.001</b>	22	-0.461	<b>&lt;0.05</b>
BD	218	-0.076	>0.05	43	0.331	<b>&lt;0.05</b>



**Figure 2.5** Weighted response ratios ( $\ln RR_{++}$ ) of soil  $N_2O$  emission (a), and  $NO_3^-$  leaching (b) to crop residue application in dependence on the composition of basic fertilizer, N fertilizer type and the application time. The mean effect and 95% CIs are shown. If the CI does not overlap with zero, the response is considered significant. Numbers in parentheses indicate the number of observations.



The  $\ln RR_{++}$  of  $N_2O$  emission and  $NO_3^-$  leaching across all the studies varied with the fertilizer components, N fertilizer types, and fertilizer application times (Table S2.1). In comparison with the control, the overall effect of synthetic fertilizer application on  $N_2O$  emissions in combination with residues application was not significant, and the response of  $N_2O$  emissions to NPK compound fertilizer and single N fertilizers was statistically similar (Fig. 2.5a). In addition, the different N forms had no significant effect on  $N_2O$  emissions when applied with crop residues (Fig. 2.5a). Fertilizer application frequencies higher than four times per growing season could mitigate  $N_2O$  emission by 31.9% incorporated with crop residue application (Fig. 2.5a, Table S2.2). Nitrogen fertilizer composition significantly controlled the effect size of crop residue application on  $NO_3^-$  leaching. Application of NPK fertilizer increased the  $\ln RR_{++}$  of  $NO_3^-$  leaching by 19.8%, whereas it was significantly decreased by 21.9% with application of single N fertilizer (Fig. 2.5a, Table S2.3). Among the different forms of synthetic N fertilizers,  $NH_4NO_3$  significantly decreased  $NO_3^-$  leaching by 23.2% (Fig. 2.5a, Table S2.3). In contrast, the effect of urea did not significantly change the effect of crop residue application on  $NO_3^-$  leaching. The analysis also revealed that when the fertilizer was applied only once during the growing season,  $NO_3^-$  leaching was significantly reduced by 58.1% (Fig. 2.5b, Table S2.3).



**Figure 2.6** Weighted response ratios ( $\ln RR_{++}$ ) of soil  $N_2O$  emission (a), and  $NO_3^-$  leaching (b) to crop residue application in dependence on residue type, tillage depth, and duration. The mean effect and 95% CIs are shown. If the CI does not overlap with zero, the response is considered significant. Numbers in parentheses indicate the number of observations.

The effect of crop residue application on soil  $N_2O$  emissions varied across residues types (Table 2.1). Application of low C:N residues (C:N < 25), but also of the high C:N residues maize straw or wheat straw significantly stimulated  $N_2O$  emission by 163.4%, 34.9% and 19.4%, respectively (Fig. 2.6a, Table S2.2). In contrast,  $N_2O$  emission decreased by 17.1%, 52.3%, and

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74.5% with rice straw, sawdust, or sugarcane straw application, respectively (Fig. 2.6a, Table S2.2). Crop residue application generally decreased  $\text{NO}_3^-$  leaching, e.g., by 19.1% with wheat straw application (Fig. 2.6b, Table S2.3).

Tillage was also found to bias the effect of crop residue application on  $\text{N}_2\text{O}$  emissions. A significant increase in  $\text{N}_2\text{O}$  emissions occurred when no-tillage or reduced tillage was performed on the top 10 cm layer (Fig. 2.6a). In addition, short experimental duration (< 1 year) was associated with a significant increase in  $\text{N}_2\text{O}$  emissions by 117.8% (Fig. 2.6a, Table S2.2). In contrast to  $\text{N}_2\text{O}$  emission, tillage and duration of crop residue application had on average no significant effect on soil  $\text{NO}_3^-$  leaching relative to the control (Fig. 2.6b).

## **2.4 Discussion**

### **2.4.1 Climatic conditions**

Our analysis revealed that crop residue application significantly stimulated  $\text{N}_2\text{O}$  emission on average by 29.7% (Table S2.2). Relative to the control, crop residue application caused an insignificant increase of  $\text{N}_2\text{O}$  emission in the tropical zone (Fig. 2.3), and the effect size of  $\text{N}_2\text{O}$  emission to crop residue return was characterized by a significantly negative linear relationship with MAT and MAP at the large scale (Table 1). This might be explained primarily by MAT and MAP being a function of climate and geographical location, which affect microbial nitrification and denitrification processes and subsequently  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching (Barnard et al., 2006; Xu et al., 2012). The high temperatures in the tropical and subtropical zone might stimulate SOM decomposition if there is enough precipitation, thereby improving N availability for nitrifiers and denitrifiers. However, the C released from crop residues might offset the N availability by stimulating soil microbial N immobilization (Sun et al., 2018). Second, labile C input could stimulate soil respiration and  $\text{O}_2$  depletion, which can cause  $\text{O}_2$  limitation in soil and thereby decrease the denitrification-related  $\text{N}_2\text{O}:\text{N}_2$  molar ratio by stimulation of complete reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Paul and Beauchamp, 1989; Vinten et al., 1998). For instance, greater  $\text{N}_2\text{O}$  emission was observed for sites with lower MAT, potentially caused by a stronger limitation of  $\text{N}_2\text{O}$  reduction by low temperature than  $\text{N}_2\text{O}$  production (Avalakki et al., 1995; Keeney et al., 1979). Third, moisture regulates soil  $\text{O}_2$  diffusion. Soil in tropical and subtropical zones with high precipitation has a higher tendency towards anoxic conditions, which foster complete denitrification with reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Davidson and Swank, 1986).

This study further revealed that crop residue application decreased soil  $\text{NO}_3^-$  leaching by 14.4% relative to the control (Table S2.3), indicating that residues application improved soil water and fertilizer-N retention capacity in accordance with Blanco-Canqui et al. (2007). Possible reasons could be on the one hand a decrease in leachate percolation (Xia et al., 2018), which leads to

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an increase in  $\text{NO}_3^-$  retention, and on the other hand an increase in cation exchange capacity (CEC) (Xia et al., 2018), which reduces the availability of free  $\text{NH}_4^+$  in the soil solution for nitrification by deprotonated carboxyl groups and thereby leads to a decrease in nitrification rate (Blanco-Canqui and Lal, 2009). A third reason might also be temporary N absorption in soil pores or N adsorption on the surface of undecomposed residues (Yang et al., 2018b). Compared with the control, no significant decrease in  $\text{NO}_3^-$  leaching after crop residue return was observed for the temperate zone. This is perhaps due to the fact that temperate soils with comparably lower MAP have a higher nitrification activity, thereby promoting the accumulation of  $\text{NO}_3^-$ . In addition, compared with the tropical and subtropical zone, the annually more evenly distributed precipitation in the temperate zone might attenuate the effect of crop residues return on  $\text{NO}_3^-$  leaching.

#### **2.4.2 Land use type**

Land use type, coupled with the availability of  $\text{O}_2$ , soil C, and N substrates, controls soil  $\text{N}_2\text{O}$  emission significantly (Butterbach-Bahl et al., 2013; Davidson et al., 2000). Our statistical results showed an opposite effect of residue return on  $\text{N}_2\text{O}$  emission between upland and paddy soil (Fig. 2.3, Table S2.2). The 18% decrease in  $\text{N}_2\text{O}$  emissions from paddy soil could be explained by increasing microbial N immobilization and complete denitrification (Aulakh et al., 2001). Compared with the control, HCA degradation accelerates the  $\text{O}_2$  consumption in rhizosphere and bulk soil. Hence, it creates an anaerobic condition, which together higher dissolved organic carbon (DOC) availability favored denitrification and a complete reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Firestone and Davidson, 1989). Yet, residue return increased  $\text{N}_2\text{O}$  emission by 46% in upland soil, which is similar to the findings of Xia et al. (2018) and Liu et al. (2014). Compared with paddy soil, the upland ecosystem has a lower moisture content, usually coupled with higher  $\text{O}_2$  availability in soil aggregates (Xia et al., 2018). Moreover, available N from residue decomposition favors autotrophic nitrification and heterotrophic denitrification, thereby increasing  $\text{N}_2\text{O}$  rather than  $\text{N}_2$  emission, in upland soil (Chen et al., 2013; Davidson et al., 2000). The responses of  $\text{NO}_3^-$  leaching to residue return were similar in upland or paddy soil (Fig. 2.3b). Residue return decreased  $\text{NO}_3^-$  leaching by reducing leachate percolation by 14% and 13% in upland and paddy soil, respectively. In upland soil, especially after residue application, soil microorganisms are forced to mine available N to keep the narrow C:N typically found for microbial biomass (Reichel et al., 2018). Moreover, higher SOC content after residue return can increase the cation exchange capacity, which prevents  $\text{NH}_4^+$  loss and reduces its availability for the conversion to  $\text{NO}_3^-$  (Blanco-Canqui and Lal, 2009).

#### **2.4.3 Soil pH**

Soil pH is an important factor regulating soil  $\text{N}_2\text{O}$  emission (Butterbach-Bahl et al., 2013). In

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our meta-analysis, crop residue return remarkably stimulated soil N<sub>2</sub>O emission. The increase in N<sub>2</sub>O emission was particularly pronounced in soils with pH 5.5-6.5 or > 7.5 (Fig. 2.4a). One potential reason could be the pH sensitivity of the enzyme N<sub>2</sub>O reductase (Bakken et al., 2012), i.e., its intolerance to low and high pH, which leads to an inhibition of the reduction of N<sub>2</sub>O to N<sub>2</sub> during denitrification at low and high pH, and hence to an increase in the mole fraction of N<sub>2</sub>O:N<sub>2</sub> (Koskinen and Keeney, 1982; Liu et al., 2010).

Compared with pH-neutral soil, higher N<sub>2</sub>O emission in moderately acidic soils could be attributed to faster lignin and cellulose degradation, which stimulates the development of nitrifier and denitrifier communities, especially in N-rich soil (Pometto and Crawford, 1986). In contrast, alkaline soil was shown to have higher N<sub>2</sub>O production potential due to the specific stimulation of AOB, associated with a high NH<sub>4</sub><sup>+</sup> oxidation rate (Law et al., 2011). Furthermore, the tendency towards higher N<sub>2</sub>O emissions at lower and higher pH could also be due to the fact that the two steps of autotrophic nitrification, i.e., the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup> by AOB and AOA, and of NO<sub>2</sub><sup>-</sup> to NO<sub>3</sub><sup>-</sup> by NOB, have differently wide optimum pH ranges, with the optimum pH range of NOB (7.9 ± 0.4) being narrower than that of AOB (8.2 ± 0.3) and AOA (7 ± 1) (Park et al., 2007; Gubry-Rangin et al., 2011). Any deviation from the optimum pH range of NOB to higher or lower values would favor the first step of nitrification, i.e., the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>2</sub><sup>-</sup>, leading to temporary NO<sub>2</sub><sup>-</sup> accumulation, which in turn can lead to substantial N<sub>2</sub>O emission (Venterea, 2007).

Soil pH is a critical factor for soil NO<sub>3</sub><sup>-</sup> leaching (Cevallos et al., 2015). Our analysis indicated that crop residue application significantly decreased NO<sub>3</sub><sup>-</sup> leaching in neutral soil in contrast to soil with pH > 7.5 (Fig. 2.4b). It is known that soil pH affects microbial nutrient immobilization and enzyme activity (Cao et al., 2016), but also the physicochemical properties of soil C-additives are crucial for the mitigation of NO<sub>3</sub><sup>-</sup> leaching. For instance, lime and wood ash increased soil pH and NO<sub>3</sub><sup>-</sup> leaching (Chinkuyu et al., 1999; Gómez-Rey et al., 2012), while biochar was found to mitigate NO<sub>3</sub><sup>-</sup> leaching despite an increase of soil pH (Knowles et al., 2011). Compared with fungi, bacteria have a comparably narrow optimum pH 6.5-7.5, which implies a higher bacterial activity and biomass in neutral soil than in acid or alkaline soil. Cellulase released by specific microorganisms stimulates the decomposition of crop residues, and the input of larger amounts of labile C enhances in turn microbial N immobilization.

#### **2.4.4 Soil texture**

Soil texture is an important factor shaping the size and distribution of soil pores and, hence, affecting soil aeration and O<sub>2</sub> availability, which are critical for decomposition of crop residues as well as the subsequent soil N transformation and loss pathways (Chen et al., 2013; Skiba and Ball, 2002; Xia et al., 2018). Soils with coarse texture and high gas permeability rapidly stimulate crop residue decomposition and microbial respiration (Chen et al., 2013). However,

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as a consequence of stimulated  $O_2$  consumption after residues incorporation, anoxic microsites might develop in the soil, which favor denitrification and  $N_2O$  emission at moderately low redox potential between 200 and 400 mV (Flessa and Beese, 1995; Yu and Patrick, 2003). In contrast, crop residue return significantly decreased  $N_2O$  emission from clay soil (clay content > 55%) (Fig. 2.4a). This might be due to the generally lower gas diffusivity in clayey soils, which decreases the decomposition rate of degradable organic residues and hence N mineralization, and which promotes even lower redox potentials than in sandy soils, i.e., low enough for  $N_2O$  reduction (Jarecki et al., 2008; Weitz et al., 2001). Furthermore, the clayey soils usually also have a higher CEC, which enhances the adsorption of  $NH_4^+$  by soil clay particles, which in turn can decrease  $NO_2^-$  production by AOB and  $NO_2^-$ -related  $N_2O$  emissions (Venterea et al., 2015). Soil  $NO_3^-$  leaching is regulated by the soil hydrologic regime. In this meta-analysis, crop residue application decreased  $NO_3^-$  leaching by 14.4% (Table S2.3). Residues application to soil stimulates microbial N retention, which leads to a decrease in  $NO_3^-$  leaching. Moreover, the straw return can also decrease  $NO_3^-$  leaching through decreased leachate percolation by increased water-holding capacity of the soil (Gu et al., 2013). However, we found that the effect of residues application on  $NO_3^-$  leaching in sandy soil was not significant (Fig. 2.4b), which might mainly be attributed to the large pore size and poor water retention capacity of sandy soil (Gaines and Gaines, 1994). In addition, the better air permeability of sandy soil is conducive to rapid decomposition of SOM and subsequent nitrification of the ammonium  $NH_4^+$  released, and together with the inhibition of anaerobic denitrification,  $NO_3^-$  accumulation and finally  $NO_3^-$  will be promoted (Gaines and Gaines, 1994).

#### **2.4.5 Synthetic fertilizer application**

Our results showed that there was no significant effect of the different components of synthetic fertilizer applied on  $N_2O$  emission. Zhou et al. (2017a) reported that globally the application of manure was associated with higher  $N_2O$  emissions than synthetic fertilizer, which is mainly due to the larger input of easily available C with manure, stimulating  $N_2O$  emission from denitrification. Compared with  $NH_4^+$  or urea, higher  $N_2O$  emission was observed for residues return combined with  $NO_3^-$  as fertilizer (Fig. 2.5a). Nitrate fertilizer can serve directly as substrate for denitrification, causing higher  $N_2O$  emission together with the easily available C released during crop residue decomposition (Senbayram et al., 2012; Xia et al., 2020).

Excessive or ill-timed application of N fertilizer can lead to an over-supply of N in the soil that cannot be compensated by microbial immobilization any more, resulting in an enhanced risk of  $N_2O$  emission (Hatfield and Cambardella, 2001). The present analysis indicated that, compared with other methods, the application frequency of N fertilizer more than four times per growing season could decrease  $N_2O$  emission in combination with crop residue return (Fig. 2.5a). As well-timed, adequate fertilization is beneficial to the direct, demand-driven use of N by plants,

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excessive N losses can be avoided by this means.

In contrast to  $\text{N}_2\text{O}$ , there was a significant difference between the fertilizer components regarding  $\text{NO}_3^-$  leaching, when jointly applied with crop residues. Compared with single N application, crop residue return combined with NPK fertilizer application increased  $\text{NO}_3^-$  leaching significantly (Fig. 2.5b). There are two possible explanations. First,  $\text{NH}_4^+$  adsorbed to the soil matrix might be substituted by  $\text{K}^+$  and released to the soil solution, and subsequently converted to  $\text{NO}_3^-$  by nitrification. Second, the concomitant application of P might alleviate or terminate a potential P limitation of nitrifiers, thereby favoring the transformation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  (Cleveland et al., 2002; Purchase, 1974).

Residues return decreased  $\text{NO}_3^-$  leaching after application of urea, albeit non-significantly (Fig. 2.5b). Urea is quickly hydrolyzed to  $\text{NH}_4^+$ , which then can be either adsorbed to the soil matrix or be quickly immobilized by soil microbial biomass, especially after residues application (Jarecki et al., 2008). In contrast, we found that  $\text{NO}_3^-$  leaching was significantly decreased when with  $\text{NH}_4\text{NO}_3$  application. One reason could be that the  $\text{NO}_3^-$  of  $\text{NH}_4\text{NO}_3$  can directly serve as substrate for denitrification, which would reduce the  $\text{NO}_3^-$  load of the soil by converting it at least partially to gaseous N forms ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ). However, there are two additional potential explanations: on the one hand, the increase in CEC caused by residues application reduces the availability of free  $\text{NH}_4^+$ , thereby limiting nitrification (Blanco-Canqui and Lal, 2009; Kim et al., 2012; Qian and Cai, 2007); on the other hand, straw enhanced microbial N immobilization due to its high C:N ratio, and by this decreased the substrate availability for nitrification and denitrification (Wang et al., 2014).

#### **2.4.6 Crop residue type**

Previous research showed that easily available C released by residue degradation stimulates soil microbial N transformation from inorganic to organic form (Chen et al., 2013; Ma et al., 2009; Shan and Yan, 2013). It is considered an efficient method to maintain soil fertility globally, though the efficiency could depend on residue type. Therefore, the potential risk of environmental pollution has to be evaluated for each crop residue type separately. It was shown previously that soil amended with crop residues with high C:N ratio stimulated microbial N immobilization, in contrast to N-rich crop residues (Baggs et al., 2000; Millar and Baggs, 2005). The increase in soil C substrate availability due to incorporation of crop residues with a high content of easily available C, such as wheat straw, in combination with high soil mineral N content can stimulate  $\text{N}_2\text{O}$  emission substantially (Yue et al., 2017). In contrast, N released through the quick decomposition of low C:N residues (C:N < 25), e.g., alfalfa and soybean, provided N in excess of the plant and microbial N demand (Shan and Yan, 2013).

Easily available C stimulates microbial growth and activity in particular, provided that the N supply is sufficient, but labile C also serves as electron donor for the reduction steps of

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denitrification from  $\text{NO}_3^-$  to  $\text{N}_2$  and supplies essential energy for heterotrophic microbial activity (Firestone and Davidson, 1989). Therefore, input of labile C to soil can have, as already discussed in the previous sections, basically two effects on  $\text{N}_2\text{O}$ , i.e., a reduction in  $\text{N}_2\text{O}$  emission due to microbial N immobilization, or an increase in  $\text{N}_2\text{O}$  emission due to stimulation of denitrification at intermediate redox potential. Based on our results, the effect size of crop residue return to soil on  $\text{N}_2\text{O}$  emission was significantly and negatively correlated with sawdust, sugarcane straw, or rice straw application (Fig. 2.6a). In contrast, wheat or maize straw stimulated  $\text{N}_2\text{O}$  emission in our analysis (Fig. 2.6b), which was also reported by Shan and Yan (2013). Large amounts of high C:N residues with low content of soluble, easily available C, like sawdust, sugarcane or rice straw, will force heterotrophic microorganisms to mine available N (Cleveland and Liptzin, 2007), thereby decreasing the N resource for nitrification and denitrification and subsequent  $\text{N}_2\text{O}$  emission (Baggs et al., 2000). Crop residues with a higher content of soluble, easily available C, like wheat or maize straw, will not only stimulate the growth of heterotrophic soil microorganisms, but also stimulate denitrification due to high  $\text{O}_2$  consumption, and hence  $\text{N}_2\text{O}$  emission (Reichel et al., 2018).

Our results also showed that wheat straw application inhibited soil  $\text{NO}_3^-$  leaching significantly, while the effect of return of other residues with different C:N ratio on  $\text{NO}_3^-$  leaching was not significant (Fig. 2.6b). This finding suggests that the C:N ratio is not the main factor affecting  $\text{NO}_3^-$  leaching, but possibly the fraction of easily available C that stimulates microbial N immobilization (see above), or perhaps either physical characteristics of crop residues that control soil  $\text{NO}_3^-$  leaching, such as increased water retention, or that particularly wheat straw stimulates denitrification due to the high amount of easily available C, thereby converting most of the  $\text{NO}_3^-$  to gaseous N forms ( $\text{N}_2\text{O}$ ,  $\text{N}_2$ ). Another possible explanation is that wheat straw can reduce  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations in the surface soil and percolating water by increasing crop N uptake, thereby decreasing  $\text{NO}_3^-$  leaching (Yang et al., 2018b).

#### **2.4.7 Tillage**

Several tillage methods in combination with crop residue return were used in the studies we analyzed. Our results showed that surface application of crop residues and shallow tillage (0-10 cm) stimulated  $\text{N}_2\text{O}$  emission significantly (Fig. 2.6a). This is possibly due to increased denitrification activity stimulated by the anoxic conditions caused by the rapid decomposition of incorporated crop residues, associated with high  $\text{O}_2$  consumption and fostered by high temperatures in the first 10 cm of the soil (Kandeler et al., 1999; Ma et al., 2009). In contrast, residues return with deep tillage (> 10 cm) caused no statistically significant difference in  $\text{N}_2\text{O}$  emission compared to the control (Fig. 2.6a). Deep tillage reduces the BD of the soil, thereby improving soil  $\text{O}_2$  availability and inhibiting denitrification (Khurshid et al., 2006).

In terms of  $\text{NO}_3^-$  leaching, we did not find a significant influence of the tillage method used for

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crop residue return (Fig. 2.6b). This might be due to the fact that soils with different texture react very differently to tillage regarding stimulation or inhibition of mineralization, nitrification, and denitrification. For instance, no significant effect of tillage on  $\text{NO}_3^-$  leaching was found for a coarse sandy soil, whereas a significant effect was observed for sandy loam soil (Hansen and Djurhuus, 1997).

#### **2.4.8 Duration of the experiments**

The duration of arable land management is a critical factor affecting the effect size of crop residue return on soil N retention. Our analyses revealed a significant difference in  $\text{N}_2\text{O}$  emission between soils with and without crop residue return, when the duration of the experiment was less than one year (Fig. 2.6a). A reason could be that the majority of C and N will be released from the residues in the first weeks and/or months, and afterwards the effect will be gradually reduced (Chen et al., 2013). Fast and substantial nutrient release from decomposing crop residues was found to stimulate nitrification and denitrification, favoring  $\text{O}_2$  depletion and the formation of partial anoxic conditions, which stimulate  $\text{N}_2\text{O}$  emission rapidly (Xia et al., 2018). However, no significant difference in  $\text{N}_2\text{O}$  emission between soils with and without crop residue incorporation was observed when the duration of the experiment was longer than one year, suggesting a potential adaptation effect of the soil and its microbial community to the treatment.

Our analysis also showed that short-term crop residue return can reduce  $\text{NO}_3^-$  leaching by more than 10%, in contrast to long-term (> 3 years) crop residue return (Fig. 2.6b). In the short term, the growing microbial biomass acts as a sink for inorganic soil N, stimulated by the increased input of labile C, thereby reducing the risk of  $\text{NO}_3^-$  leaching (Zechmeister-Boltenstern et al., 2002). On the contrary, long-term crop residue return might lead to saturation of the SOC pool with subsequent adaptation of the microbial community to this new equilibrium, thereby on the one hand increasing its resistance against disturbance or environmental change, but on the other hand also decreasing its N buffering capacity (Griffiths and Philippot, 2013).

#### **2.4.9 Overall effects of residue return on N losses**

So far, some mitigation effects of crop residue return on N runoff were reported (Blanco-Canqui et al., 2006; Xia et al., 2018). The phenomenon could be attributed to the change of soil structure, which leads to an increase in water infiltration rate and a decrease in surface runoff, and thereby to a decreased risk of soil erosion (Lindstrom, 1986). Recently, some new perspectives were also presented that crop residue return can increase  $\text{NH}_3$  emission by stimulating ammonium-related soil N transformations. For example, Xia et al. (2018) found that crop residue return significantly increased the gross N mineralization rate by 82.4% and dissimilatory  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  (DNRA) by 155%. The stimulation of these specific N transformation



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processes leads to an increase in soil  $\text{NH}_4^+$  content, which in turn serves as substrate for  $\text{NH}_3$  emission.

#### **2.4.10 Potential publication bias**

We collected data with wide geographic coverage to achieve high robustness of this meta-analysis. The results of funnel plot analysis and Egger's indicator test showed that there was no systematic publication bias in our database. In parallel, we checked the geographic coordinates of the outliers in the funnel plot and found that they were not located in the southern hemisphere (Fig. S2.3). Therefore, we conclude that the inclusion of data from poorly studied areas of the world did not result in publication bias. Nevertheless, we acknowledge that the lack of data in some areas of the world warrants more intensive study, particularly in the southern hemisphere.

### **2.5 Conclusion**

Overall, this meta-analysis provides valuable insights into the effect of crop residue return on soil  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching and their dependence on climate zone, soil properties, and arable land management. We present two major perspectives: First, crop residue application increases soil  $\text{N}_2\text{O}$  emission by stimulating microbial nitrification and denitrification. Second, soil  $\text{NO}_3^-$  leaching is mitigated by crop residue amendment. Our results reveal some opposing trends when compared with previous studies and provide new guidance for future research. Crop residues need to be applied depending on soil fertility and climatic conditions. For instance, amendment of nutrient-poor soil with low C:N residues is recommended, thereby decreasing the application of synthetic fertilizer, accelerating the recovery of soil fertility and supplying nutrients for the next growing season, especially in areas with low crop yield. Besides, crop residue return combined with deep tillage should be generally applied based on site-specific soil conditions because  $\text{N}_2\text{O}$  emission and N losses through leaching, runoff, or  $\text{NH}_3$  volatilization, which pose a risk of soil nutrient loss without safeguarding procedures, are thereby minimized. However, due to differences in soil structure and microbial activity between different soils and sites, the determination of the optimal tillage frequency requires further study. Overall, the focus should be on harnessing the positive effects of crop residue return for maintaining and improving soil fertility and for sustaining or even increasing crop productivity. Ultimately, this will help to balance sustainable farming, economic benefit, and protection of the environment in the future.

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## **Chapter 3**

### **Effect of C:N:P stoichiometry on soil nitrous oxide emission and nitrogen retention**

Based on:

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### 3.1 Introduction

Nitrogen is crucial for obtaining high crop yields, but also bears the risk of N losses via  $\text{NH}_3$  and  $\text{N}_2\text{O}$  emission, and via  $\text{NO}_3^-$  leaching into groundwater (Zhu and Chen, 2002; Iqbal et al., 2009). The GWP of  $\text{N}_2\text{O}$  is estimated to be 298 times higher than that of  $\text{CO}_2$ , and it severely accelerates  $\text{O}_3$  depletion in the stratosphere (Ravishankara et al., 2009). Due to agricultural intensification, atmospheric  $\text{N}_2\text{O}$  concentrations have increased to 331.1 ppb in 2018, which is 23% higher than the pre-industrial level (WMO, 2020). Agricultural management, in particular fertilization with N, is responsible for almost 60% of the anthropogenic  $\text{N}_2\text{O}$  emission and contributes about 21% to the annual global  $\text{N}_2\text{O}$  emission (IPCC, 2021). The global demand for N in crop production was 110 million tons in 2015 and is predicted to further increase in the future (Lu and Tian, 2017). Currently, farmers tend to overuse N fertilizers to maintain high crop yields, which results in the accumulation and loss of inorganic N. Hence, alternative N management strategies need to be developed and applied to reconcile soil N availability with high crop yield and quality on the one side, and mitigation of N losses on the other side (Zhang et al., 2015; Bowles et al., 2018).

One common way to immobilize excess N in agricultural soils is application of organic C. Organic amendments such as wheat straw, maize straw and leonardite (Table S3.3) with large C content have been known for a long time to induce N immobilization by promoting microbial biomass growth (Ocio et al., 1991; Pan et al., 2017; Bashir et al., 2018; Reichel et al., 2018). However, application of organic C to soils frequently leads also an increase in  $\text{N}_2\text{O}$  emissions (Zhou et al., 2017a). Despite this knowledge, more research is needed to optimize N immobilization by microbial biomass and minimize N release via  $\text{N}_2\text{O}$  emission. For example, labile C released from wheat straw in high amounts not only favors microbial N uptake, but can also act as electron donor for reductions step of denitrification of  $\text{NO}_3^-$ , a major source of  $\text{N}_2\text{O}$  emission (Firestone and Davidson, 1989).

Types of HCA with C:N ratios above 60 and with a large fraction of microbially decomposable cellulose and hemicellulose stimulate microbial uptake of excess N from soil to maintain their narrow C:N ratio of about 7 (Cheshire et al., 1999). Wheat straw (C:N 50-100), for instance, contains a large fraction of easily decomposable organic C, which caused a rapid N retention in microbial biomass of up to  $42 \text{ kg N ha}^{-1}$  when applied at a rate of  $4.5 \text{ t C ha}^{-1}$ , with the caveat that under the optimal microbial growth conditions in the laboratory the N retention lasted only several weeks (Reichel et al., 2018). Other HCA, such as leonardite and sawdust as byproducts of lignite mining and wood processing, respectively, have promising properties beneficial to increasing the C and N storage in the soil over a longer period. Leonardite (i.e., an oxidized form of lignite) is characterized by similar C:N ratios as wheat straw, but in addition contains humic acids and a smaller fraction of easily available C (Fowkes and Frost, 1960). Research

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into the effects of leonardite amendment on arable soil and crop performance has been widely conducted, and largely positive results have been reported. For instance, leonardite application modified soil quality and increased the SOC and TN contents of soil (Sariyildiz, 2020; Wang et al., 2020), accompanied by increased crop yield and fertilizer utilization rate (Akinremi et al., 2000; Akimbekov et al., 2020; Sariyildiz, 2020). Furthermore, leonardite was found to enhance tolerance to drought and P deficiency stress of specific plants (Kaya et al., 2020). Spruce sawdust with its very large C:N ratio of about 400 has a large fraction of C bound in lignin, which makes it less decomposable for bacteria, but potentially favors fungal growth and N immobilization in the long term (Vano et al., 2011; Reichel et al., 2018). Hence, leonardite and sawdust in combination with wheat straw could further improve the N immobilization capacity in soil. However, sawdust is widely used as renewable fuel source and for production of wood particle boards, and leonardite as a fossil fuel is not a renewable resource, suggesting that it should not be recommended for wide application in agriculture. Therefore, we used leonardite and sawdust in our study only as model substances to compare their efficacy in immobilizing N with that of wheat straw, and to study their characteristics as well as short- and longer-term effects in soil, especially on N retention, which could open avenues to new, engineered organic soil amendments that are based on renewable resources.

Phosphorus as an essential element for biological growth and activity may temporarily and locally limit microbial N immobilization (Mori et al., 2010; Mehnaz and Dijkstra, 2016). Generally, the P effects are moderated by biotic and abiotic factors such as soil texture, aeration condition, initial nutrient status, and the availability of labile C. However, despite the considerable number of studies on this aspect, the results have been ambiguous so far. For instance, Sundareshwar et al. (2003) demonstrated that P fertilizer application increased N immobilization and mitigated N<sub>2</sub>O emission in South Carolina (USA). Correspondingly, Hall and Matson (1999) found a 10-100 times greater N<sub>2</sub>O emission in “P-limitation” than “N-saturated” soil, which had been amended with N fertilizer. These results revealed that P shortage limited microbial N immobilization and increased the risk of soil N losses, both via NO<sub>3</sub><sup>-</sup> leaching or N<sub>2</sub>O emission. In contrast, Mori et al. (2010) found that P addition increased soil N<sub>2</sub>O emission in a tropical evergreen broad-leaved forest as a result of N supply to nitrifying and denitrifying microorganisms, indicating that P availability might be an important factor for both HCA-derived N retention and N<sub>2</sub>O emission. Hence, assessing the potential response of soil N retention to different P and C availabilities, especially for different soil conditions, is of great relevance for sustainable agricultural development.

The overall aim of this study was to improve the knowledge on C:N:P stoichiometry effects in combination with HCA amendment of soils. We hypothesized that co-application of HCA with increasing amounts of P would increase N retention and lower N<sub>2</sub>O emission. Our specific research questions were: (1) Which of the selected HCA types will be the most effective to

retain N in agricultural soil, (2) can N retention be increased by P co-application, and (3) do the selected soils with different pH values respond in the same way to the application of HCA and P?

## 3.2 Materials and methods

### 3.2.1 Soils and organic amendments

Three different soils spanning a pH range of two pH units were chosen for the experiment. Nutrient-poor silty soil (PUS) with a pH of 7.6 was sampled in February 2018 at the Inden lignite mine (50.89° N, 6.34° E) close to Jülich (Nordrhein-Westfalen, Germany) a few weeks after the soil-loess mixture had been re-deposited after mining for later agricultural recultivation. Nutrient-rich silty agricultural soil (RUS) with pH 6.4 was sampled in the same area, but before mining, in September 2018 from a nearby agricultural field after harvest of winter wheat (50.85° N, 6.41° E). Six soil cores (5 cm diameter, 0–15 cm depth) were taken randomly from each field, mixed, and sieved to 2 mm to get a composite soil sample free of visible crop residues. The soils were air-dried and stored at room temperature until the start of the experiment. Nutrient-rich sandy soil (RSS) with a pH of 5.5 was obtained as fresh substrate from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA, Speyer, Germany) and treated in the same way. Table 3.1 shows a summary of the initial physicochemical properties of the three soil substrates. Winter wheat straw was obtained from the Hohenschulen experimental farm, operated by the University of Kiel, Germany. Spruce sawdust (art. no. 823 “Siebgutfein”) was obtained from HolzRuser, Bornhöved, Germany. Leonardite was obtained from RWE Power AG, Köln, Germany. Detailed information on each HCA is given in Table 3.2.

**Table 3.2** Mean values (and unit) of soil parameters of carbon- and nutrient-poor silty soil (PUS), carbon- and nutrient-rich silty soil (RUS), and carbon- and nutrient-rich sandy soil (RSS): microbial biomass C (MBC), pH (0.01 mol CaCl<sub>2</sub>), soil organic carbon (SOC), total nitrogen (TN), carbon-to-nitrogen ratio (C:N), ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), easily extractable P (P<sub>ex</sub>), maximum water holding capacity (WHC), bulk density (BD), and soil texture.

Soil	MBC (mg kg <sup>-1</sup> )	pH	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	C:N	NH <sub>4</sub> <sup>+</sup> (mg N kg <sup>-1</sup> )	NO <sub>3</sub> <sup>-</sup> (mg N kg <sup>-1</sup> )	P <sub>ex</sub> (mg kg <sup>-1</sup> )	WHC (%)	BD (g cm <sub>3</sub> <sup>-1</sup> )	Texture
PUS	15.4	7.6	2.7	0.6	3	<0.8	0.9	13.2	21.5	1.3	Loam
RUS	119.6	6.4	10.7	1.1	10	<0.8	11.2	57.2	51	1.4	Silt loam
RSS	103.0	5.5	7.5	0.6	13	<0.8	10.4	39.3	38	1.4	Loamy sand

**Table 3.3** Mean values ( $\pm$  standard deviation) of the organic carbon (TOC), total nitrogen (TN), and C:N ratio of the high organic carbon amendment (HCA) leonardite (oxidized lignite), winter wheat straw, and spruce sawdust.

HCA	TOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	C:N ratio	Lignin (%)
Wheat Straw	436 $\pm$ 1.8	9.7 $\pm$ 0.3	45 $\pm$ 1	17
Sawdust	440 $\pm$ 1.2	1.6 $\pm$ 0.1	275 $\pm$ 16	25
Leonardite	463 $\pm$ 2.1	9.2 $\pm$ 0.3	50 $\pm$ 1	

### 3.2.2 Experimental design

All types of soil received 50 mg N kg<sup>-1</sup> as calcium ammonium nitrate. Aliquots of each soil received HCA in form of wheat straw, sawdust, or leonardite at a rate of 1550 mg C kg<sup>-1</sup>. The soil was further aliquoted and then amended with 0 (P<sub>0</sub>), 140 (P<sub>140</sub>), or 250 (P<sub>250</sub>) mg P kg<sup>-1</sup> in form of triple superphosphate. Each of three treatment replicates comprised 6 g of 2-mm sieved and air-dried soil. Soil was mixed with N, P and HCA homogenously, placed in 22-ml gas chromatography (GC) vials (20 mm in diameter and 70 mm in height) and compacted to a BD of 1.35 g cm<sup>3</sup>. Control soil only received the according amount of N and P, but no HCA. In order to set the WHC to 60% and to reactivate microbial activity, deionized water was dripped evenly onto the surface of each soil sample 24 h before the start of the experiment. The experiment was conducted at room temperature and comprised 42 incubation days as described below.

### 3.2.3 Greenhouse gas measurements

Soil samples were incubated in open GC vials. Only for gas measurement the vials were closed gas-tight with a septum and lid, and opened again after each GC measurement. The N<sub>2</sub>O and CO<sub>2</sub> emission was analyzed in the vial headspace on incubation days 1, 3, 7, 14, 21, 28 and 42 by using a GC-ECD/FID (Clarus 580, PerkinElmer, Rodgau, Germany) in intervals of 1, 4, 7 and 10 h as described in Reichel et al. (2017). Water loss from open GC vials between the GC measurements was monitored by weekly weighing, and water content was readjusted if needed. A linear regression slope was used to calculate the N<sub>2</sub>O and CO<sub>2</sub> emission rate with the following formula:

$$F = \frac{\frac{\Delta C}{\Delta t} \times V \times T_0 \times M}{m \times T_a \times V_m} \quad (1)$$

where  $F$  represents the gas emission flux;  $\Delta C/\Delta t$  represents the change of gas concentration in ppmv for CO<sub>2</sub> and ppbv for N<sub>2</sub>O;  $V$  represents the headspace volume in liter;  $M$  represents the molar mass of N in N<sub>2</sub>O or C in CO<sub>2</sub>, respectively;  $m$  represents the amount of soil in g dry

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weight;  $V_m$  represents the molar volume of ideal gases ( $22.414 \text{ l mol}^{-1}$ ) at  $0^\circ\text{C}$  and  $101.325 \text{ kPa}$ , corrected for the gas sample temperature using  $T_\theta$  ( $273.15 \text{ K}$ ) and  $T_a$  (air temperature in  $\text{K}$ ) (Reichel et al., 2018).

### 3.2.4 Soil extraction and analysis

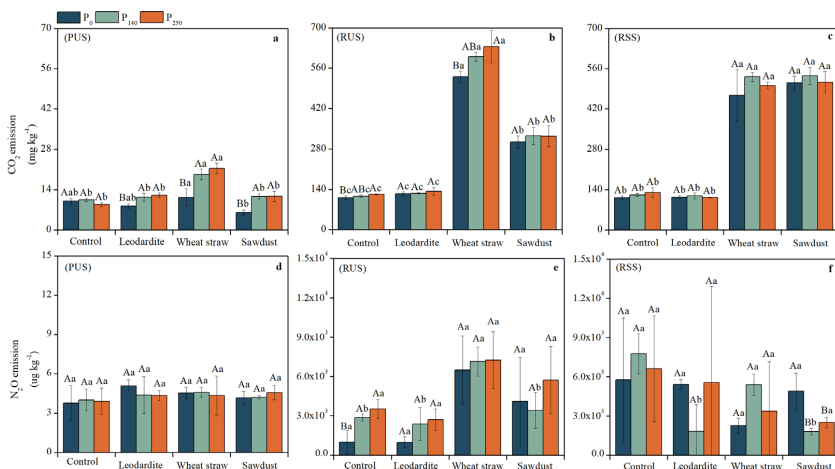
Extractable mineral N ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$ ),  $\text{P}_{\text{ex}}$ , and DOC were simultaneously extracted with  $0.01 \text{ M}$  calcium chloride ( $\text{CaCl}_2$ ), using a soil-to-solution ratio of  $1:10 \text{ (w/v)}$ . Soil slurries were shaken horizontally at  $200 \text{ rpm}$  for  $2 \text{ h}$ , centrifuged at  $690 \text{ RCF}$  (relative centrifugal  $g$  force) for  $15 \text{ min}$ , filtered through syringe filters ( $25 \text{ mm}$ ,  $0.45 \mu\text{m}$  polypropylene membrane syringe filter, VWR Europe). Samples were stored at  $4^\circ\text{C}$  before measurement. Easily extractable P was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCA 7600, Thermo Fisher Scientific, Oberhausen, Germany). Nitrate,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$  were measured with ion chromatography (DX-500, Dionex, USA). Dissolved organic carbon was measured with a TOC analyzer (TOC-VcPH + TNM-1 + ASI-V, Shimadzu, Japan). Microbial biomass carbon (MBC) was determined with the chloroform fumigation-extraction method as described by Reichel et al. (2017).

### 3.2.5 Statistical analysis

Stepwise multiple regression was performed to test the effect of HCA and P co-application on soil parameters and  $\text{CO}_2$  or  $\text{N}_2\text{O}$  emissions. One-way ANOVA was used to test the soil HCA and P treatments for significant differences. The differences were considered significant when  $P < 0.05$ . All statistical analyses were performed using  $21.0 \text{ SPSS}$  software package for Windows.

### 3.3 Results

#### 3.3.1 CO<sub>2</sub> emission



**Figure 3.7** Mean values and standard deviation of CO<sub>2</sub> and N<sub>2</sub>O emissions after 42 days of incubation for the control, the different types of high organic carbon amendments (HCA; wheat straw, sawdust, leonardite), and the phosphorus (P) co-application levels (P<sub>0</sub>, P<sub>140</sub>, P<sub>250</sub>) in nutrient-poor silty soil (PUS), nutrient-rich silty soil (RUS), and nutrient-rich sandy soil (RSS). Different uppercase letters denote significant differences between the P co-application levels within the same treatment (control or HCA). Different lowercase letters denote significant differences between the types of HCA within the same P co-application level at  $P < 0.05$ .

Overall, the factors soil type, HCA, and incubation time affected the CO<sub>2</sub> emission rates significantly (Table S3.1). The PUS control emitted about 10 times less CO<sub>2</sub> compared to RUS and RSS (Fig. 3.1a, b, c). In PUS, P co-application was a significant factor influencing CO<sub>2</sub> emission rates (Table S3.2, Fig. S3.1d, g, j). Furthermore, amendment with wheat straw significantly increased cumulative CO<sub>2</sub> emission in combination with P co-application in PUS (Fig. 3.1a). In RUS, both wheat straw and sawdust, co-applied with P, enhanced cumulative CO<sub>2</sub> emissions relative to the control (Fig. 3.1b). In RSS, wheat straw likewise increased the CO<sub>2</sub> emission rates during the incubation (Fig. S3.1i). Interestingly, sawdust applied to RSS had the same effect, reaching a CO<sub>2</sub> emission level comparable to that of wheat straw (Fig. S3.1i, l).

#### 3.3.2 N<sub>2</sub>O emission

Overall, the soil N<sub>2</sub>O emission rate was significantly affected by soil type, HCA, and incubation time (Table S3.1). In the PUS control, the N<sub>2</sub>O emission rate was generally between 5,000 and

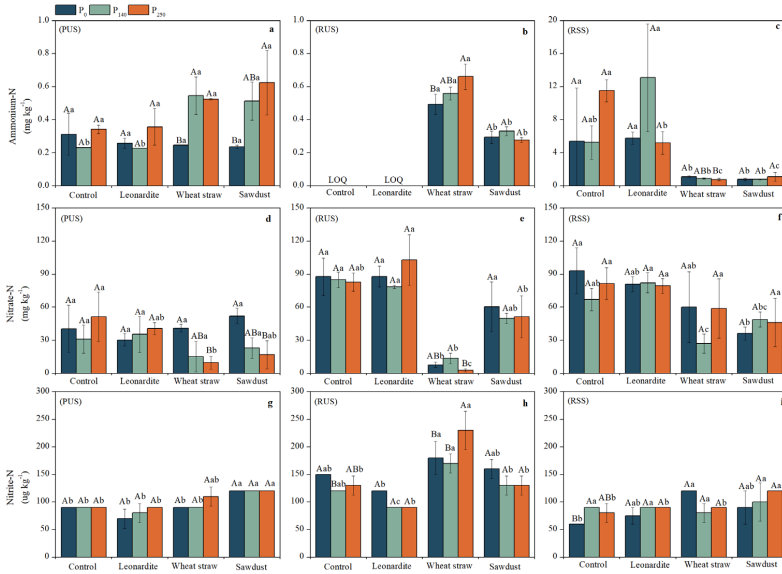


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10,000 times lower than in the corresponding RUS and RSS control (Fig. S3.2a-c). Within the first seven incubation days, P co-application affected the N<sub>2</sub>O emission rate significantly in several soils and treatments. For example, in RSS amended with sawdust P co-application significantly reduced N<sub>2</sub>O emission rates (Fig. S3.2l). In contrast, P co-application to RUS significantly increased cumulative N<sub>2</sub>O emissions in the control, but not in combination with the different HCA types (Fig. 3.1e). Wheat straw applied to RUS resulted in significantly larger cumulative N<sub>2</sub>O emissions, whereas leonardite and sawdust applied to RUS did not significantly affect the cumulative N<sub>2</sub>O emission (Fig. 3.1e). P140 co-applied to the RSS treatments with leonardite and sawdust significantly reduced cumulative N<sub>2</sub>O emission compared with the control and the wheat straw treatment (Fig. 3.1f). Nonetheless, in most treatments, as most conspicuously visible for PUS, effects of P co-application disappeared at the level of cumulative N<sub>2</sub>O emissions over the entire incubation period of 42 days (Fig. 3.1d). This is also reflected in the results of the multiple regression analysis, which revealed that P co-application to HCA was not significantly affecting N<sub>2</sub>O emission rates when the whole observation period of 42 days was analyzed and all treatments were aggregated (Table S3.2).

### **3.3.3 Extractable mineral N**

In PUS amended with wheat straw or sawdust, more NH<sub>4</sub><sup>+</sup> was retained after 42 days compared to the control and the leonardite treatment, but only at the intermediate P co-application level P<sub>140</sub> (Fig. 3.2a). In RUS, NH<sub>4</sub><sup>+</sup> retention was also significantly higher in soil amended with sawdust and wheat straw compared to the control and the leonardite treatment (Fig. 3.2b). Here, the NH<sub>4</sub><sup>+</sup> concentration in the RUS wheat straw treatment was significantly larger than in the sawdust treatment (Fig. 3.2b). In RSS, the NH<sub>4</sub><sup>+</sup> concentration was about ten times larger compared to PUS and RUS (Fig. 3.2a-c). Opposite to RUS, NH<sub>4</sub><sup>+</sup> concentrations in RSS were significantly higher in the control and the leonardite treatment than in the according wheat straw and sawdust treatments (Fig. 3.2b-c).



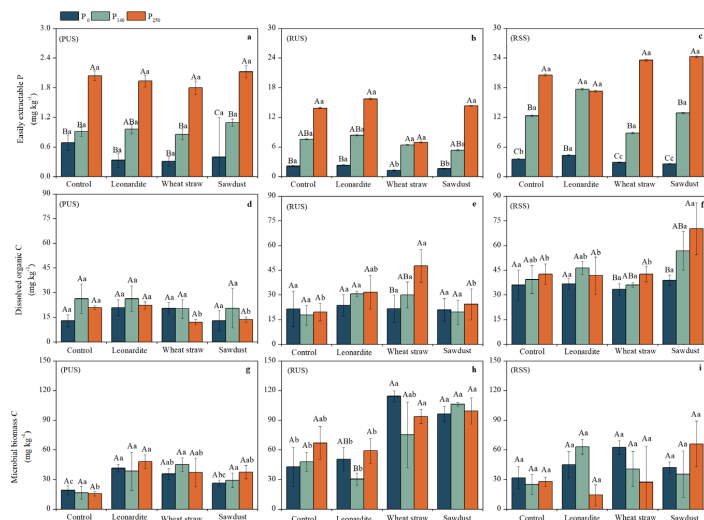
**Figure 3.8** Mean values and standard deviation of ammonium, nitrate, and nitrite in the soil after 42 days of incubation for the control treatment, the treatments with different types of HCA (wheat straw, sawdust, leonardite), and P co-application levels ( $P_0$ ,  $P_{140}$ ,  $P_{250}$ ) in PUS, RUS, and RSS. Different uppercase letters denote significant differences between the P co-application levels within the same treatment (control or HCA). Different lowercase letters denote significant differences between the types of HCA within the same P co-application level at  $P < 0.05$ . LOQ denotes the limit of quantitation

In PUS, the  $\text{NO}_3^-$  concentration was significantly reduced when sawdust or wheat straw was applied in combination with additional P, whereas it was not changed by addition of leonardite or in combination with additional P compared to the control (Fig. 3.2d). In RUS, less  $\text{NO}_3^-$  was found in soil amended with sawdust and significantly less from wheat straw-amended soil compared to the control and leonardite treatment (Fig. 3.2e). In RSS, wheat straw and sawdust had a comparable, but less significant effect on  $\text{NO}_3^-$  concentrations (Fig. 3.2f). In PUS and RUS, wheat straw application resulted in the lowest  $\text{NO}_3^-$  concentration at the highest P co-application level ( $P_{250}$ ) (Fig. 3.2d-e), while in RSS, the lowest  $\text{NO}_3^-$  concentration was found in the wheat straw treatment at  $P_{140}$  (Fig. 3.2f).

Generally, the  $\text{NO}_2^-$  concentration was larger in RUS compared to the PUS and RSS treatments (Fig. 3.2g-i). In the RUS wheat straw treatment at P co-application level  $P_{250}$ , a significantly larger  $\text{NO}_2^-$  concentration was determined than in the control (Fig. 3.2h). In RSS, wheat straw significantly increased  $\text{NO}_2^-$  concentration without P co-application compared to the control, but not after addition of P (Fig. 3.2i).

### 3.3.4 Extractable P, dissolved organic C and microbial biomass C

Easily extractable P generally increased with the P co-application level (Fig. 3a-c). In PUS,  $P_{ex}$  was about ten times lower compared to RUS and RSS (Fig. 3a-c). In the  $P_0$  treatments of RUS and RSS, wheat straw and sawdust significantly lowered  $P_{ex}$  compared to the according control or leonardite treatment (Fig. 3b-c). There was no significant difference in  $P_{ex}$  between the  $P_{140}$  and  $P_{250}$  treatment of RUS with wheat straw and RSS with leonardite (Fig. 3b, c). Lower DOC was found in PUS after amendment with wheat straw and sawdust at P co-application level  $P_{250}$  compared to the control and leonardite treatment (Fig. 3d). The largest DOC concentration was found at the highest P co-application level  $P_{250}$  in RUS with wheat straw and in RSS with sawdust (Fig. 3e-f). The PUS control had the lowest MBC compared to all other treatments, but MBC generally increased after addition of HCA (Fig. 3g). In RUS, wheat straw and sawdust most significantly increased MBC compared to the control and leonardite treatment (Fig. 3g-i). In contrast, a comparable increase of MBC was not observed for the RSS treatments (Fig. 3h, i), and the responses of MBC to additional P were not significant.



**Figure 3.9** Mean values and standard deviation of easily extractable P, dissolved organic C, and microbial biomass C in the soil after 42 days of incubation for the control treatment, the treatments with different types of HCA (wheat straw, sawdust, leonardite), and P co-application levels ( $P_0$ ,  $P_{140}$ ,  $P_{250}$ ) in the PUS, RUS, and RSS. Different uppercase letters denote significant differences between the P co-application levels within the same treatment (control or HCA). Different lowercase letters denote significant differences between the types of HCA within the same P co-application level at  $P < 0.05$

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### 3.4 Discussion

Amendment of soil with HCA to improve soil fertility and nutrient availability is common practice worldwide, which might directly and/or indirectly impact soil N retention through biotic and abiotic factors, such as soil temperature and moisture, nutrient availability, soil microorganisms (Lu et al., 2009).

Promoting microbial biomass growth and activity will increase N transformation from mineral to organic state, thereby decreasing the risk of N losses and severe environmental pollution, e.g. atmospheric aerosol pollution due to  $\text{NH}_3$  emissions and groundwater eutrophication due to  $\text{NO}_3^-$  leaching (Zhu and Chen, 2002; Zhang et al., 2015). In line with our hypothesis, co-application of P and wheat straw, and to a lesser extent sawdust, increased microbial biomass and  $\text{NH}_4^+$  retention in PUS and RUS (Fig. 3.2a-b; Fig. 3.3g-h). These results of soil N retention amended with HCA were consistent with other lab or field studies (Yang et al., 2015; Reichel et al., 2018; Xia et al., 2018). Labile C derived from the application of decomposable residues in silty soil stimulated microbial growth and activity. Simultaneously, restricted  $\text{O}_2$  availability might inhibit nitrification, and might even result in the quick dieback of aerobic microbial biomass (Bollmann and Conrad, 1998).

Nutrient-rich sandy soil treated with wheat straw and sawdust had a lower  $\text{NH}_4^+$  content than the control (Fig. 3.2c). This might partially be due to the comparably higher nitrification activity, sandy texture and aeration condition. Previous studies reported that wheat straw application enhanced the immobilization of  $\text{NO}_3^-$  (Cheng et al., 2012; Reichel et al., 2018). Furthermore, in comparison with the control, co-application of HCA and P reduced the amount of leachable  $\text{NO}_3^-$  (Fig. 3.2d-f). There are two possible underlying mechanisms for these observations: First, microbial growth was stimulated by the input of easily available C, but the microbes were forced at the same time to take up available N from soil in order to maintain their C:N ratio (Cleveland and Liptzin, 2007). Second,  $\text{O}_2$  depletion due to increased microbial activity might have impeded nitrification, but stimulated denitrification at the same time, which in turn resulted in the decrease of  $\text{NO}_3^-$  content (Bollmann and Conrad, 1998).

Decomposability of HCA is a critical factor controlling the microbial C resource. Compared with spruce sawdust and leonardite, wheat straw with a C:N ratio around 60 has a comparably greater decomposability in the short term (Fowkes and Frost, 1960; Cheshire et al., 1999; Reichel et al., 2018). In our experiment, wheat straw application effectively stimulated MBC in RUS (Fig. 3.3h), as released C from wheat straw increased the efficacy of this HCA to retain N and mitigated fast microbial N re-mineralization. Similarly, wheat straw was also found to stimulate soil microbial growth and activity in the period after crop harvest (Reichel et al., 2018). In contrast, HCA with high C:N ratio and lower decomposability might retain N in the soil at a lower level, but keep it for a longer period, by sustained C release and N immobilization.

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For instance, spruce sawdust has a large fraction of C bound in lignin, which makes it less decomposable to bacteria but potentially favors fungal growth (Vano et al., 2011; Reichel et al., 2018). Leonardite, characterized by a recalcitrant humic fraction, is available at large quantities as a byproduct of lignite mining, and demonstrated a great potential of improving soil fertility and yield in the long term (Yolcu et al., 2011).

Already minor changes in SOM content cause a profound effect on microbial biomass and activity, which is associated with the consumption of O<sub>2</sub> and emission of CO<sub>2</sub>, but also frequently of N<sub>2</sub>O (Manlay et al., 2007; Zhou et al., 2017a; Reichel et al., 2018). Our experiment suggested that RUS developed a significant denitrification activity after wheat straw application (Fig. 3.1e), while most likely nitrification was stimulated in RSS (Fig. 3.1f), associated with lower N<sub>2</sub>O emissions. This is partially due to differences in aeration as a result of differences in soil texture, emphasizing that the effect of HCA addition on mitigation of soil N losses is not only depending on the physicochemical properties of the applied HCA, but also on the soil properties, mainly texture, pH and nutrient status (Reichel et al., 2018; Wang et al., 2018). However, the advantages of increased N retention by HCA in our study were achieved at the cost of increased CO<sub>2</sub> emissions in most of the treatments. Only PUS exhibited a clearly lower CO<sub>2</sub> emission rate than RUS and RSS, even when the same HCA was applied (Fig. S3.1). This was very likely due to the initial very low SOC and nutrient content as well as the very low microbial biomass level of PUS, associated with the inhibition of SOM mineralization due to restricted microbial growth (Chen et al., 2014), and is also in accordance with the concept by Bredebecke et al. (1993), who considered soil CO<sub>2</sub> emission as an effective indicator of soil quality, i.e. biological activity.

In our study, a strong decrease in CO<sub>2</sub> emission was observed 3-7 days after the start of incubation (Fig. S3.1). Obviously, soil microorganisms quickly consumed the C released from the HCA, especially from wheat straw (Reichel et al., 2018). In contrast to wheat straw, sawdust contains recalcitrant lignin, usually associated with low decomposability (Kostov et al., 1991). However, we found a greater CO<sub>2</sub> emission from RSS than PUS and RUS treated with sawdust (Fig. 3.1a-c), which might have been caused by the better aeration of RSS compared to the two silty soils, favoring bacterial and fungal HCA decomposition, probably by extracellular laccases and peroxidases of ligninolytic bacteria and fungi which require a good aeration status of the soil (Janusz et al., 2017). In contrast, the effect of sawdust application on CO<sub>2</sub> emission was insignificant in PUS when compared with the control (Fig. 3.1a). But again, this was very likely due to the very low initial microbial activity in this soil.

Soil nutrient availability and microbial biomass control the decomposition of SOM through alleviating potential microbial nutrient deficiencies and allowing the microbial biomass to achieve the stoichiometric C:N:P ratios required for decomposition (Cleveland et al., 2006; Chen et al., 2014). Therefore, addition of HCA with P co-application might have the potential

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to stimulate CO<sub>2</sub> emission in nutrient-poor soil, implying that the alleviation of P limitation of decomposers together with HCA and N application would be accompanied by an increase in C release (Mori et al., 2010). In our experiment, P co-applied with wheat straw stimulated the C loss via CO<sub>2</sub> from both silty and sandy soils (Fig. 3.1a-c), similar to the study of Hui et al. (2020) who demonstrated that P is a critical factor enhancing CO<sub>2</sub> emissions in tropical forest soil together with labile C substrate. Moreover, exogenous C from degrading wheat straw alleviated the C deficiency limiting soil microbial CO<sub>2</sub> emission in P-rich soil.

Soil nitrification and denitrification are complex processes, controlled by the availability of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and SOM, as well as affected by soil aeration status, soil moisture and soil temperature (Smith, 2017). Application of HCA could directly or indirectly affect cropland soil N retention through altering biotic and abiotic factors (Ma et al., 2007; Zhang and Marschner, 2017; Reichel et al., 2018; Wang et al., 2018). For instance, incorporation of wheat straw stimulated gross NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> immobilization rates and inhibited gross nitrification rate in a cultivated black chernozem (Cheng et al., 2012). However, the application of organic amendment can also have negative effects, i.e. too fast release of easily available C might not reduce N<sub>2</sub>O emission from nitrification, but increase N<sub>2</sub>O emission from denitrification by fast depletion of the O<sub>2</sub> pool during the degradation of C (Zhou et al., 2017b). Hence, it is necessary to evaluate the potential risk of N loss via N<sub>2</sub>O emission after HCA application. Our results showed that N<sub>2</sub>O emission rate continuously decreased after HCA application (Fig. S3.2). This could be probably explained in part by the autotrophic nature of soil nitrifying bacteria, in that the depletion of NH<sub>4</sub><sup>+</sup> induced a decline in bacterial nitrification in spite of the ample CO<sub>2</sub> emission during the incubation.

N<sub>2</sub>O emission via denitrification might have been a potential additional source of N<sub>2</sub>O (Maag and Vinther, 1996). Especially the wheat straw treatment of RUS as well as the wheat straw and sawdust treatments of RSS (Fig. 3.1b-c), which caused the highest CO<sub>2</sub> emission and, therefore, also the highest O<sub>2</sub> consumption, might have led to conditions favoring denitrification, at least in part of the soil. It has been observed previously that N<sub>2</sub>O formation after straw addition to soil is very likely a tightly coupled nitrification-denitrification process (Wu et al., 2017). This is reflected in the tendency towards lower NO<sub>3</sub><sup>-</sup> content in those treatments and soils at the end of the experiment, which might have been caused by NO<sub>3</sub><sup>-</sup> consumption by denitrification.

In general, wheat straw has a comparably faster decomposition potential and releases larger quantities of easily available C for microbial biomass growth than sawdust and leonardite. The large amount of easily available C from wheat straw quickly stimulates microbial biomass growth and might create anaerobic hotspots in the soil, which in turn trigger denitrification associated with additional N<sub>2</sub>O emission (Wu et al., 2017; Reichel et al., 2018), which was particularly visible in RUS (Fig. 3.1e). In RSS, leonardite and sawdust application decreased N<sub>2</sub>O emission at the intermediate P level in RSS (Fig. 3.1f), despite the strong stimulation of

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CO<sub>2</sub> emission by sawdust, which was similar to the wheat straw treatment in that soil (Fig. 3.1e). This observation remains difficult to explain in view of the fact that leonardite did not stimulate CO<sub>2</sub> emission, i.e., aerobic microbial activity in RSS, while sawdust did. A possible explanation might be a complex interplay between biological, chemical and physical processes in the different soils amended with the different HCA that triggered several abiotic processes (such as adsorption and desorption and redox reactions) as well as biotic processes, such as microbial N immobilization, nitrification, denitrification, nitrifier denitrification or coupled nitrification-denitrification. Also the difference in pH between RUS and RSS by one unit might play an important role here in determining which of the microbial species prevail and which processes dominate. However, with the data available it is not possible to disentangle the complex processes, which demand for further scrutiny in dedicated experiments.

Soil P availability has been shown to affect N<sub>2</sub>O emission through microbial biomass growth and activity. However, there is still no uniform conclusion so far. Some studies report that P addition can decrease N<sub>2</sub>O emission by stimulating microbial N retention (Hall and Matson, 1999; Sundareshwar et al., 2003), while others found greater N losses via N<sub>2</sub>O in response to P addition in field experiments (Mori et al., 2010; Zhang et al., 2014; Mehnaz and Dijkstra, 2016). In our experiment, P co-applied with wheat straw stimulated N<sub>2</sub>O emission from RUS, while sawdust applied to RUS and RSS reduced the amount of emitted N<sub>2</sub>O (Fig. 1e-f). Mori et al. (2010) found that P application stimulated N<sub>2</sub>O and NO emissions from soils of an *Acacia mangium* plantation. This was partially confirmed by our result in that P application significantly increased N<sub>2</sub>O emission in the RUS control (Fig. 3.1e), implying that P addition stimulated the activity of nitrifiers and denitrifiers by alleviating the P deficiency in RUS. On the contrary, P co-applied with sawdust inhibited N<sub>2</sub>O emission from RSS (Fig. 3.1f), which was consistent to findings of Sundareshwar et al. (2003), who found that improved soil P availability in coastal wetland soil decreased the rate of N<sub>2</sub>O production via denitrification by alleviating P limitation of heterotrophic microorganisms.

### 3.5 Conclusion

This study explored the potential effects of HCA applied at different P levels and soil conditions on soil N retention. We found that the initial soil nutrient status was a decisive factor for the degradation of HCA and that soil N dynamics were strongly controlled by the availability of C and P. The application of wheat straw had the largest N retention effect compared to sawdust and leonardite, especially in silty soil. P co-applied with HCA might further reduce mineral N loss, especially from poor (marginal) soils, and can be beneficial for further reduction of N<sub>2</sub>O emissions, but with a strong dependence on the type of soil and the P application rates. Overall, application of HCA has great potential for improving soil N retention, but more research is needed to fully elucidate the interactions between organic matter quality, soil physicochemical

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properties, N and P availability, microbial activity, and  $\text{NO}_3^-$  and gaseous N losses. Only then targeted agricultural management options towards optimization of agricultural nitrogen use efficiency involving HCA and adjusted N and P fertilization can be developed.



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## Chapter 4

### **Effect of combined addition of high-carbon amendments with phosphorus fertilizer and $^{13}\text{C}$ -labelled glucose on soil N retention and greenhouse gas emissions**

Based on:

Li Z, Reichel R, Wissel H, Zhao K, Brüggemann N. Effect of combined addition of high-carbon amendments with phosphorus fertilizer and  $^{13}\text{C}$ -labelled glucose on soil N retention and greenhouse gas emissions. *Front. Soil Sci.* (under review)

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## 4.1 Introduction

Nitrogen is one of the primary nutrients in ecosystems, which is involved in many biological activities and closely related to soil quality, climate change, and plant productivity. The global N demand for crop production in 2015 was 110 million tons and further increased to nearly 119 million tons in 2020 (Lu and Tian, 2017). Furthermore, the United Nations estimated that the global population will increase to 9.1 billion by 2050 (McNicoll, 2006). Hence, farmers will have to increase the N application amount, especially in Africa, to fulfill the increasing demand for crop products. However, only 10-65% of applied fertilizer-N is utilized by crops, and N fertilization rates usually have a negative relationship with N use efficiency beyond a certain threshold (Cassman et al., 1998). As a result, the excess N fertilizer in soil causes environmental pollution through the emission of  $\text{N}_2\text{O}$  and  $\text{NH}_3$ , and even more through leaching of  $\text{NO}_3^-$  (Said-Pullicino et al., 2014).

Nitrous oxide is an important GHG and is currently the third-largest contributor to global warming after  $\text{CO}_2$  and  $\text{CH}_4$ . In addition, it has a 298 times higher GWP than  $\text{CO}_2$  in a 100-year timeframe (Stocker, 2014). Since the industrial revolution, mainly as a result of mineral fertilizer application, the atmospheric  $\text{N}_2\text{O}$  concentration has increased to 334.8 ppb in 2022, which is 23% higher than the pre-industrial level (Stocker, 2014). Moreover, agriculture is responsible for almost 60% of the anthropogenic  $\text{N}_2\text{O}$  emission and contributes about 21% to the annual global  $\text{N}_2\text{O}$  emission (Stocker, 2014). The availability of soil N can also affect soil C sequestration by affecting the microbial community and the production of GHG simultaneously (Xia et al., 2014). For instance, increased availability of N may stimulate the decomposition of humus to fulfill the C demand of microbes (C mining), resulting in higher  $\text{CO}_2$  emission (Chu et al., 2007). In addition, besides N fertilizers, the residues of nutrient-rich crops, such as sugar beet, legumes and oilseed rape, are also a potential N source after decomposition (Reichel et al., 2018). Hence, appropriate nutrient control strategies for arable land, and especially how to increase the soil N use efficiency, are urgently needed.

The addition of labile C is regarded as an effective method to promote SOM decomposition. Zhang et al. (2019) reported that the priming effect caused by the addition of glucose significantly increased MBC and promoted decomposition of both labile and recalcitrant SOM in a mixed forest in Dinghushan Natural Reserve, Guangdong, China. Furthermore, Wild et al. (2014) demonstrated a positive effect of glucose addition on SOM decomposition even in arctic permafrost soil. Numerous studies showed that, induced by the release of labile C from SOM decomposition, soil microorganisms are forced to immobilize mineral N to keep their C:N ratio around 7 (Cleveland and Liptzin, 2007; Reichel et al., 2018; Scheller and Joergensen, 2008). In general, HCA with C:N ratios above 60 contain large fractions of relatively easily available C in form of cellulose and hemicellulose, which can be used by microorganisms for growth

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(Kamau et al., 2021). Lal (2005) estimates the amount of crop residues produced globally at  $2.8 \times 10^9$  Mg year<sup>-1</sup> for cereal crops, implying that N retention induced by HCA application may be used specifically to improve the N use efficiency of crop rotations. A meta-analysis showed that straw return significantly increased SOC content on average by 14.9% and TN by 9.8% (Xia et al., 2014). However, most of the positive effects of N retention only last for a few weeks or months due to the fast decomposition of wheat straw and diminish quickly afterwards. Compared with wheat straw, sawdust as the byproduct of wood processing with a C:N ratio of up to 400 contains more recalcitrant compounds, which are characterized by lower microbial degradation rates, and thus slower N immobilization due to the low C availability at least within the first weeks after sawdust addition (Reichel et al., 2018). However, the slower decomposition of sawdust or similar substrates could extend the positive effect of HCA application on soil microbial N retention (Kostov et al., 1991; Van Kuijk et al., 2017). Therefore, combined application of sawdust with readily degradable organic substances, such as glucose, may close the gap in N immobilization in the first month after application by accelerating microbial N immobilization, while providing C for prolonged retention of N later. It has to be emphasized, though, that sawdust is widely used as a renewable fuel source and for the production of wood particle boards, suggesting that it should not be recommended for wide application in agriculture. Therefore, we used sawdust in our study only as a model substance to investigate the effect of more recalcitrant, high C:N materials, on soil N retention.

Phosphorus is a crucial component of DNA, phospholipids, and ATP, and other essential molecules in all living organisms. The availability of P in soil affects soil biogeochemical processes, together with the development of microbial biomass and community composition and the decomposition of SOC (Brookes et al., 1984). Hence, an imbalanced C:N:P stoichiometric ratio will limit microbial growth, activity and C utilization.

The objective of our study was to optimize soil N retention over a simulated winter period and re-release under spring temperature conditions through the addition and stimulated decomposition of HCA without increasing GHG emissions and N leaching. We hypothesized that 1) simultaneous application of glucose and P fertilizer would improve the N retention potential of recalcitrant HCA such as sawdust through stimulating HCA decomposition (i.e., priming), reflected in increased CO<sub>2</sub> emission, and 2) at the same time reduce N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> loss due to enhanced microbial immobilization of mineral N in the soil.

## **4.2 Materials and methods**

### **4.2.1 Soil characteristics**

Soil low in organic C, N and P was randomly sampled from six positions on a reclaimed agricultural field (0-30 cm soil depth) near the Inden lignite mine (50.88°N, 6.37°E) (Li et al.,

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2021a). The mean annual temperature is 10°C and the average rainfall in the area is 765 mm yr<sup>-1</sup>. Soil samples were pooled and mixed before passing through a 2-mm sieve. Any visible crop residues were removed from the soil, then the soil was air-dried and stored at room temperature until the start of the experiment. The basic properties of the soil substrate were: pH: 7.5 (CaCl<sub>2</sub>), NH<sub>4</sub><sup>+</sup>-N: 0.14 mg kg<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>-N: 0.46 mg kg<sup>-1</sup>, P<sub>ex</sub> (extracted with 0.01 M CaCl<sub>2</sub>): 16.50 mg kg<sup>-1</sup>, SOC: 0.35 g kg<sup>-1</sup>, TN: 0.05 g kg<sup>-1</sup>.

#### 4.2.2 Experimental design

The experiment was conducted in a full-factorial, completely randomized design of one N level × two P levels × six C amendments: 1) one N level: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added at a rate of 22 mg N kg<sup>-1</sup> dry soil, equivalent to 60 kg N ha<sup>-1</sup>; 2) two P fertilizer levels: 0 and 33 mg P kg<sup>-1</sup> dry soil, equivalent to 0 or 90 kg P ha<sup>-1</sup>; 3) six C amendments: without C addition, glucose, wheat straw, poplar sawdust, wheat straw + glucose, and sawdust + glucose. The experimental design resulted in twelve treatments in total, which were: 1) control with N, but without P and C amendment, 2) phosphorus only (P), 3) wheat straw (WS), 4) wheat straw + phosphorus (WS+P), 5) sawdust (SD), 6) sawdust + phosphorus (SD+P), 7) glucose (G), 8) glucose + phosphorus (G+P), 9) wheat straw + glucose (WS+G), 10) wheat straw + glucose + phosphorus (WS+G+P), 11) sawdust + glucose (SD+G), 12) sawdust + glucose + phosphorus (SD+G+P). Wheat straw and sawdust were applied at a rate of 1.55 g C kg<sup>-1</sup> dry soil, equivalent to 4190 kg C ha<sup>-1</sup>, and glucose was added at a rate of 0.31 g C kg<sup>-1</sup> dry soil, which was 1/5 of the total C content of wheat straw and sawdust. This ratio of glucose addition is considered optimal to avoid excessive growth of microbial biomass and changes in microbial community structure (Mehnaz et al., 2019). All twelve treatments were replicated three times.

The additives (NH<sub>4</sub><sup>+</sup>, P fertilizer, glucose, HCA) were ground and mixed into the soil individually for each replicate. The air-dry soil (350 g each) was transferred into PVC columns (diameter: 5 cm, height: 23 cm) and compacted to a BD of 1.35 g cm<sup>-3</sup>. In order to determine the incorporation of glucose in microbial biomass, <sup>13</sup>C-labelled glucose (20 atom% <sup>13</sup>C) was added (Sigma-Aldrich Pty Ltd, affiliate of Merck KGaA, Darmstadt, Germany). To reactivate the microbial activity, soil samples were rewetted to 60% WHC 24 h before the start of the experiment. All samples were placed in an incubator to simulate the local air temperature dynamics from September to April. The incubation temperatures were adjusted based on the monthly average temperatures of the winter months of the past five years in the soil sampling area: 15.2°C (September), 12.9°C (October), 7.1°C (November), 5.9°C (December), 3.4°C (January), 4.1°C (February), 6.8°C (March), and 10.2°C (April). Plastic bowls filled with water were placed in the incubator to reduce soil moisture loss. The soil moisture level of 60% WHC was maintained by replacing the weight loss weekly with deionized water, which was added evenly onto the surface of each soil sample.

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### 4.2.3 GHG sampling and flux calculation

GHG fluxes were measured on incubation days 1, 2, 3, 5, and 7 in the first week, and then weekly until the end of the incubation experiment with an infrared laser gas analyzer (G2508, Picarro, Inc., Santa Clara, CA, USA). Immediately before the measurements, the soil samples were removed from the incubator and placed on the laboratory bench next to the analyzer. A headspace chamber with tubing connected to the analyzer and an additional vent tube was placed gas-tight on top of the PVC column with the soil sample. The headspace chamber was connected to the gas analyzer in closed-loop mode, allowing the quantification of the change in CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentration over a period of 10 min (Cao et al., 2021). After each measurement, the soil columns were immediately placed back in the incubator, so that the temperature change in the soil column was negligible. Flux values were considered valid if the linear regression of the concentration increase yielded an  $R^2 > 0.81$ . In this case the linear regression slope was used to calculate the CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O flux rate with the following formula:

$$F = \frac{\frac{\Delta C}{\Delta t} \times V \times T_0 \times M}{m \times T_a \times V_m}$$

where F represents the gas emission flux;  $\Delta C/\Delta t$  represents the change of gas concentration in ppmv for CO<sub>2</sub> and ppbv for CH<sub>4</sub> and N<sub>2</sub>O; V represents the headspace volume in liter; M represents the molar mass of N in N<sub>2</sub>O or C in CO<sub>2</sub> or CH<sub>4</sub>, respectively, in g mol<sup>-1</sup>; m represents the amount of soil in g dry weight; V<sub>m</sub> represents the molar volume of ideal gases (22.414 L mol<sup>-1</sup>) at 0°C and 101.325 kPa, corrected for the gas sample temperature using T<sub>0</sub> (273.15 K) and T<sub>a</sub> (air temperature during the measurements in K) (Reichel et al., 2018). Cumulative emissions were calculated as the sum of the areas of the trapezoids formed by the two respective flux rate values of adjacent measurement dates and the time difference as the base.

### 4.2.4 Soil sampling and measurement

Soil was sampled with a custom-made stainless-steel corer (diameter: 8 mm, height: 10 cm) from top to bottom on days 7, 15, 30, 60, 105, 150, 195, and 240 after the start of the experiment from the same column. By using such a small corer, the disturbance of the soil column was minimized. Soil samples were stored at 4°C before measurement. Soil microbial biomass carbon and nitrogen (MBN) were determined with the chloroform fumigation-extraction method as described in Li et al. (2021a), and the microbial C and N was corrected with the MBC and MBN correction factors  $k_{ec} = 0.45$  and  $k_{en} = 0.54$ , respectively (Joergensen, 1996). The <sup>13</sup>C content in the fumigated and non-fumigated extracts (0.01 M CaCl<sub>2</sub>) was freeze-dried, weighed in tin capsules and then analyzed with an elemental analyzer coupled to an isotope ratio mass spectrometer (Isoprime, Elementar, Langenselbold, Germany). The <sup>13</sup>C content

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(atom%) in microbial biomass ( $^{13}\text{C}_{\text{mic}}$ ) was calculated according to the following equation:

$$^{13}\text{C}_{\text{mic}} = (C_f \times ^{13}\text{C}_f - C_{\text{nf}} \times ^{13}\text{C}_{\text{nf}}) / (C_f - C_{\text{nf}})$$

where  $C_f$ ,  $^{13}\text{C}_f$ ,  $C_{\text{nf}}$  and  $^{13}\text{C}_{\text{nf}}$  are the total amount of C and  $^{13}\text{C}$  atom% of the fumigated and non-fumigated extracts, respectively (Mehnaz et al., 2019).

To determine soil  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , DOC, and  $\text{P}_{\text{ex}}$ , fresh soil equivalent to 5 g dry soil was mixed with 0.01 M  $\text{CaCl}_2$  in 50-mL falcon tubes using a soil-to-solution ratio of 1:10 (w/v) (VWR International, Darmstadt, Germany). The soil suspensions were shaken horizontally at a speed of 200 turns  $\text{min}^{-1}$  for 90 min, then centrifuged at relative centrifugal force of  $690 \times g$  for 20 min, and filtered through syringe filters (25 mm, 0.45  $\mu\text{m}$  polypropylene membrane syringe filter, VWR Europe). Extractable P was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, iCA 7600, Thermo Fisher Scientific, Germany). Nitrate and  $\text{NH}_4^+$  were measured with ion chromatography (DX-500, Dionex, USA). Dissolved organic C in the extract solution was determined with a TOC analyzer (TOC-VcPH + TNM-1 + ASI-V, Shimadzu, Japan).

#### 4.2.5 Statistical analysis

Repeated measures ANOVA (rmANOVA) was performed to test the effects of HCA, glucose, P fertilizer, and their interactions on GHG emissions, soil nutrients, microbial biomass, and the microbial uptake of  $^{13}\text{C}$ . One-way ANOVA was conducted to test for significant differences between each measured parameter. The differences were considered significant when  $P < 0.05$ . All statistical analyses were performed using SPSS Statistics 21.0 (IBM Deutschland GmbH, Ehningen, Germany).

### 4.3 Results

#### 4.3.1 Effect of HCA, glucose, and P fertilizer addition on GHG emissions

The  $\text{CO}_2$  flux was significantly affected by HCA and glucose, respectively (Table 4.1). Carbon dioxide fluxes were generally increased in the first week of the experiment (Fig. 4.1A). The highest  $\text{CO}_2$  flux ( $1187.9 \text{ mg m}^{-2} \text{ h}^{-1}$ ) was from the WS+G+P treatment, while the control treatment ( $5.8 \text{ mg m}^{-2} \text{ h}^{-1}$ ) had the lowest  $\text{CO}_2$  flux during the incubation (Fig. 4.1A). After one month of incubation, the SD treatment had a  $\text{CO}_2$  flux comparable to the WS treatment (Fig. 4.1A). The differences between treatments gradually diminished over time (Fig. 4.1A). Compared to the control, application of HCA significantly increased cumulative  $\text{CO}_2$  emissions (Fig. 4.1D). Wheat straw and sawdust application increased the cumulative  $\text{CO}_2$  emission by 86% and 92%, respectively, compared to the control, but the difference between WS and SD was insignificant (Fig. 4.1D). Furthermore, there were no significant differences in cumulative  $\text{CO}_2$  emissions between samples with or without glucose addition for the same HCA and P

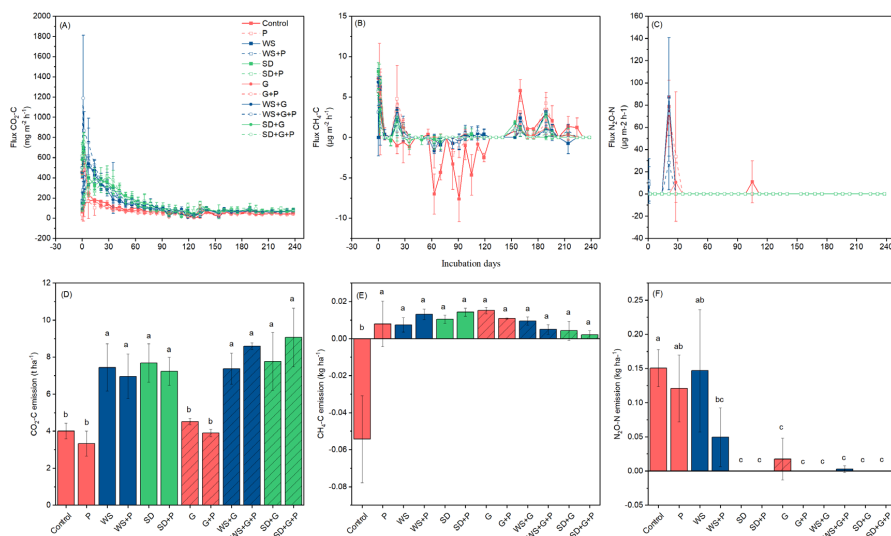
fertilizer conditions (Fig. 4.1D).

**Table 4.4** Results of repeated measures ANOVA for the main effects of high carbon amendment (HCA: WS or SD), phosphorus (P), glucose (G), and their interactions on fluxes of CO<sub>2</sub>-C, CH<sub>4</sub>-C, and N<sub>2</sub>O-N.

Treatment	CO <sub>2</sub> -C			CH <sub>4</sub> -C			N <sub>2</sub> O-N		
	<i>P</i>	<i>F</i>	Eta <sup>#</sup>	<i>P</i>	<i>F</i>	Eta <sup>#</sup>	<i>P</i>	<i>F</i>	Eta <sup>#</sup>
P	0.472	0.533	0.022	<b>&lt;0.01</b>	11.517	0.291	0.804	0.063	0.002
G	<b>&lt;0.001</b>	43.746	0.646	<b>&lt;0.05</b>	5.752	0.170	<b>&lt;0.001</b>	20.761	0.426
P * G	0.066	3.717	0.134	<b>&lt;0.001</b>	28.051	0.501	0.939	0.006	0.001
WS	<b>&lt;0.001</b>	107.532	0.870	0.162	2.146	0.118	0.301	1.143	0.067
WS * P	0.072	3.721	0.189	<b>&lt;0.05</b>	5.187	0.245	0.505	0.465	0.028
WS * G	0.666	0.194	0.012	<b>&lt;0.01</b>	12.817	0.445	0.285	1.225	0.071
WS * P * G	0.172	2.041	0.113	<b>&lt;0.05</b>	5.187	0.267	0.193	1.843	0.103
SD	<b>&lt;0.001</b>	85.561	0.842	0.102	3.014	0.159	<b>&lt;0.01</b>	15.205	0.487
SD * P	0.234	1.531	0.087	<b>&lt;0.05</b>	6.160	0.278	0.911	0.013	0.001
SD * G	0.472	0.542	0.033	<b>&lt;0.001</b>	23.737	0.597	<b>&lt;0.01</b>	12.285	0.434
SD * P * G	0.409	0.719	0.043	<b>&lt;0.01</b>	8.782	0.354	0.618	0.258	0.016

WS: wheat straw; SD: sawdust; <sup>#</sup>Eta = Partial eta squared is a measure for the effect size of a variable, calculated as the ratio of variance associated with an effect, plus that effect and its associated error variance.

Methane fluxes in the treatments were comparably low, with slightly positive CH<sub>4</sub> fluxes in the initial and final phase of the experiment, whereas the fluxes were close to zero in between (Fig. 4.1B). The highest CH<sub>4</sub> flux (8.1 µg m<sup>2</sup> h<sup>-1</sup>) occurred in the SD treatment three days after the start of the experiment. In contrast, the control was characterized by negative fluxes (CH<sub>4</sub> uptake) especially in the intermediate phase of the experiment, with the lowest single CH<sub>4</sub> flux value of -7.6 µg m<sup>2</sup> h<sup>-1</sup> three months after the start of the experiment (Fig. 4.1B) and a cumulative CH<sub>4</sub> emission of -0.05 kg ha<sup>-1</sup> (Fig. 4.1E). The application of HCA, P fertilizer, or glucose significantly increased the cumulative CH<sub>4</sub> emission compared to the control (Fig. 4.1E), and the CH<sub>4</sub> flux was significantly affected by glucose, P fertilizer, and their interaction with HCA (Table 4.1). Glucose in combination with SD and SD+P decreased the CH<sub>4</sub> flux by 40% and 53%, respectively, compared with SD and SD+P without glucose, but the difference was insignificant (Fig. 4.1E).



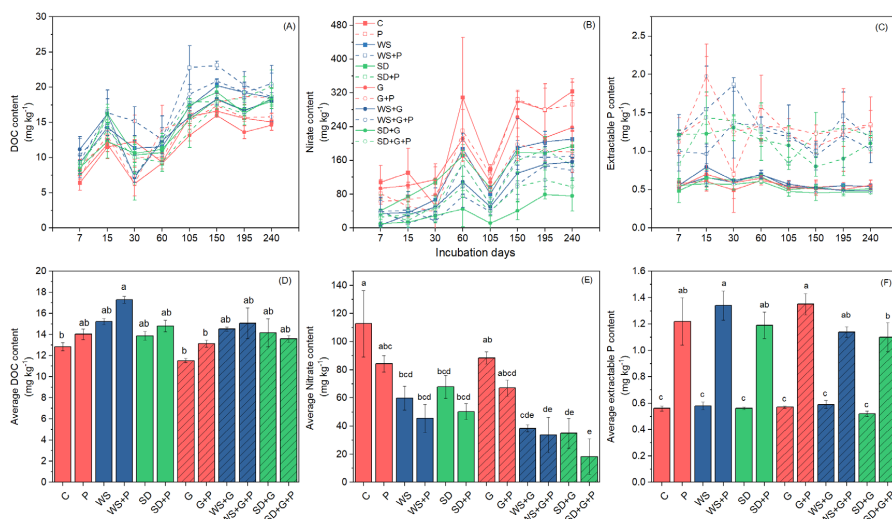
**Figure 4.10** The fluxes of CO<sub>2</sub>-C (A), CH<sub>4</sub>-C(B), N<sub>2</sub>O-N (C) during the incubation experiment and cumulative emissions of CO<sub>2</sub>-C (D), CH<sub>4</sub>-C (E), N<sub>2</sub>O-N (F) at the end of the incubation experiment (after eight months). P: phosphorus, WS: wheat straw, SD: sawdust, G: glucose. Different lowercase letters indicate a significant difference between treatments at  $P < 0.05$ .

Sawdust, glucose, and their interaction affected the N<sub>2</sub>O flux significantly during the incubation experiment (Table 4.1). The highest single N<sub>2</sub>O flux during the experiment ( $87.3 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) occurred in the WS treatment 21 days after start of the incubation, directly followed by the control and the P treatment (Fig. 4.1C). In all other treatments, N<sub>2</sub>O fluxes were much lower or even not detectable. This is also reflected in comparable cumulative N<sub>2</sub>O emissions of control, P and WS, while the cumulative N<sub>2</sub>O emissions of WS+P and G were significantly lower than the control, whereas all other treatments were zero or close to zero (Fig. 4.1F).

### 4.3.2 Effect of HCA, glucose, and P fertilizer addition on soil nutrient status

Application of HCA, P fertilizer and glucose individually affected the soil DOC content significantly (Table 4.2). Soil DOC content generally increased during the experiment, while HCA application further increased the DOC content (Fig. 4.2A). The maximum DOC content was  $23.1 \text{ mg kg}^{-1}$  in WS+P, while the minimum DOC content was  $6.3 \text{ mg kg}^{-1}$  in G+P (Fig. 4.2A). At the end of the experiment the DOC content had increased between 63% (WS+G) and 135% (control) compared to the DOC content at the start of the incubation (Fig. 4.2A). Furthermore, only the average DOC content of the WS+P treatment was significantly higher compared to HCA-free treatments (Fig. 4.2D).





**Figure 4.11** Dynamics of soil DOC (A), nitrate (B), extractable P (C), and the average content of DOC (D), nitrate (E), and extractable P (F) during the incubation experiment. P: phosphorus, WS: wheat straw, SD: sawdust, G: glucose. Different lowercase indicates a significant difference between treatments at  $P < 0.05$ .

Application of HCA, glucose, and P fertilizer significantly affected the soil  $\text{NO}_3^-$  content in this soil (Table 4.2). The  $\text{NO}_3^-$  content continuously increased after application of  $\text{NH}_4^+$  at the beginning of the experiment (Fig. 4.2B). Compared to the control, HCA application lowered the average  $\text{NO}_3^-$  concentration in soil significantly (Fig. 4.2E). The control and P treatment showed higher  $\text{NO}_3^-$  concentrations by the end of the incubation, which arrived at 323.7 and 292.3 mg kg<sup>-1</sup>, while SD+G had the lowest  $\text{NO}_3^-$  content during most of the incubation period (Fig. 4.2B). Phosphorus addition decreased the soil  $\text{NO}_3^-$  content by 20% and 24% in WS+P and SD+P, respectively, until the end of the experiment in relation to the corresponding treatments without P, i.e., WS and SD (Fig. 4.2B). In addition, the average  $\text{NO}_3^-$  content of WS, WS+P, SD, and SD+P decreased by 26%, 20%, 61%, and 34%, respectively, after glucose addition (Fig. 4.2E).

**Table 4.5** Results of repeated measures ANOVA for the main effects of high carbon amendment (HCA: WS or SD), phosphorus (P), glucose (G), and their interactions on dissolved organic carbon (DOC), nitrate (NO<sub>3</sub><sup>-</sup>), and extractable P (P<sub>ex</sub> = extracted with 0.01 M CaCl<sub>2</sub>).

Treatment	DOC			NO <sub>3</sub> <sup>-</sup>			P <sub>ex</sub>		
	<i>P</i>	F	Eta <sup>#</sup>	<i>P</i>	F	Eta <sup>#</sup>	<i>P</i>	F	Eta <sup>#</sup>
P	<0.001	19.540	0.449	<0.01	10.720	0.309	<0.001	300.469	0.926
G	<0.001	21.563	0.473	<0.001	43.214	0.643	0.286	1.192	0.047
P * G	0.056	4.025	0.144	<0.05	5.731	0.193	<0.001	64.703	0.729
WS	<0.001	117.213	0.88	<0.001	94.088	0.855	0.677	0.18	0.011
WS * P	0.874	0.026	0.002	0.111	2.853	0.151	0.317	1.068	0.063
WS * G	0.445	0.613	0.037	0.645	0.22	0.014	<0.05	6.30	0.283
WS * P * G	0.063	3.986	0.199	0.885	0.022	0.001	<0.05	6.271	0.282
SD	<0.001	25.791	0.617	<0.001	97.188	0.859	<0.05	5.38	0.252
SD * P	<0.05	6.373	0.285	<0.05	7.054	0.306	<0.001	89.339	0.848
SD * G	0.218	1.642	0.093	0.227	1.581	0.09	0.082	3.433	0.177
SD * P * G	0.065	3.940	0.198	0.13	2.17	0.119	<0.001	82.792	0.838

<sup>#</sup>Eta = Partial eta squared is a measure for the effect size of a variable, calculated as the ratio of variance associated with an effect, plus that effect and its associated error variance.

The interaction between HCA, glucose, and P fertilizer affected the soil P<sub>ex</sub> content significantly (Table 4.2). The P<sub>ex</sub> content of all soil treatments showed no dynamics, and the content was similar at the start and end of the experiment (Fig. 4.2C). Treatments with P fertilizer had significantly higher mean P levels than the treatment without P addition (Fig. 4.2F).

### 4.3.3 The effect of HCA, glucose, and P fertilizer addition on soil microbial biomass

Addition of HCA, glucose and P fertilizer significantly affected the soil MBC and MBN content, but not the MBC:MBN ratio (Table 4.3). The MBC increased during the incubation experiment in all treatments and in the control (Fig. 4.3A). HCA treatments further increased the MBC content compared to the control. The highest MBC content was found in WS+G, which reached 281.1 mg kg<sup>-1</sup> by the end of the incubation period (Fig. 4.3A). Addition of P to WS+G significantly decreased the average MBC content by 28% compared to WS+G (Fig. 4.3D). Oppositely, glucose application enhanced the average soil MBC content of WS, WS+P, SD, and SD+P by 23%, 7%, 3%, and 35%, respectively (Fig. 4.3D).

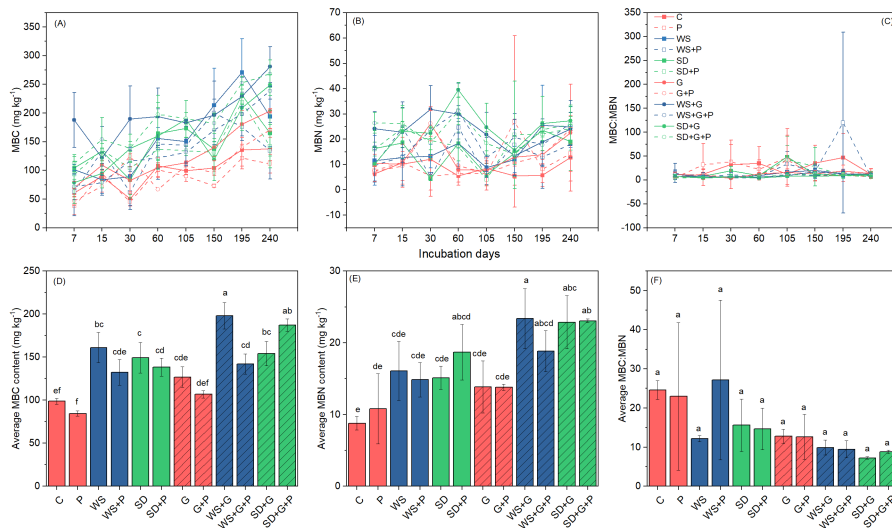
The MBN content was quite variable in all experimental variants (Fig. 4.3B). HCA application tended to increase MBN content, albeit in most cases not significantly (Fig. 4.3B). Average MBN was significantly higher than the control only in WS+G, WS+G+P, SD+P, SD+G and SD+G+P, but there was no significant difference in soil MBN content between the

corresponding wheat straw and sawdust treatments, no matter whether P fertilizer or/and glucose were added or not (Fig. 4.3E). Glucose addition increased the average MBN content, even significantly in the case of WS/WS+G by 45% (Fig. 4.3E).

**Table 4.6** Results of repeated measures ANOVA for the main effects of high carbon amendment (HCA: WS or SD), phosphorus (P), glucose (G), and their interactions on MBC, MBN, and MBC:MBN.

Treatment	MBC			MBN			MBC:MBN		
	<i>P</i>	<i>F</i>	Eta <sup>#</sup>	<i>P</i>	<i>F</i>	Eta <sup>#</sup>	<i>P</i>	<i>F</i>	Eta <sup>#</sup>
P	<b>&lt;0.01</b>	8.752	0.354	0.954	0.004	0.000	0.494	0.490	0.030
G	<b>&lt;0.001</b>	20.513	0.562	<b>&lt;0.01</b>	14.899	0.482	0.059	4.134	0.0205
P * G	0.910	0.013	0.001	0.410	0.717	0.043	0.828	0.048	0.003
WS	<b>&lt;0.001</b>	66.238	0.869	<b>&lt;0.01</b>	13.944	0.582	0.382	0.836	0.077
WS * P	0.113	3.017	0.232	0.343	0.991	0.09	0.704	0.153	0.015
WS * G	0.721	0.135	0.013	0.341	1.001	0.091	0.832	0.047	0.005
WS * P * G	0.294	1.227	0.109	0.687	0.172	0.017	0.352	0.951	0.087
SD	<b>&lt;0.001</b>	99.285	0.908	<b>&lt;0.001</b>	30.493	0.753	0.09	3.515	0.26
SD * P	<b>&lt;0.05</b>	5.621	0.36	0.845	0.04	0.004	0.843	0.041	0.004
SD * G	0.508	0.471	0.045	0.181	1.969	0.165	0.919	0.011	0.001
SD * P * G	0.107	3.134	0.239	0.537	0.408	0.039	0.816	0.057	0.006

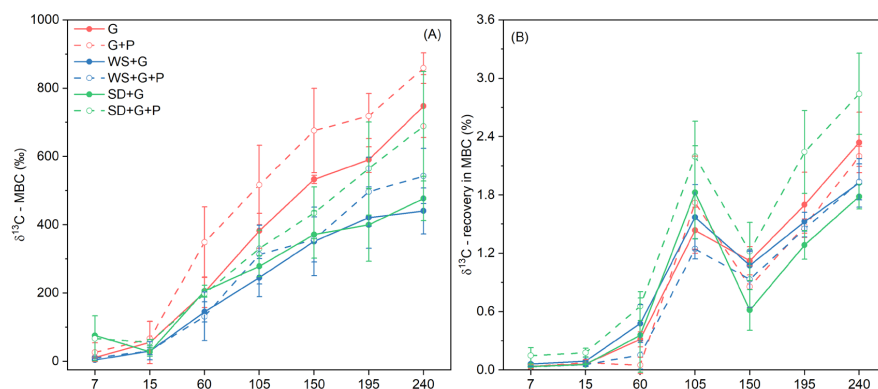
WS: wheat straw; SD: sawdust; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; <sup>#</sup>Eta = Partial eta squared is a measure for the effect size of a variable, calculated as the ratio of variance associated with an effect, plus that effect and its associated error variance.



**Figure 4.12** Dynamics of MBC (A), MBN (B), MBC:MBN (C), and the average content of MBC (D), MBN (E), and MBC:MBN (F) during the incubation experiment P: phosphorus, WS: wheat straw, SD: sawdust, G: glucose. Different lowercase indicates a significant difference between treatments at  $P < 0.05$ .

The MBC:MBN ratios in the different treatments did not fluctuate significantly during the experiment (Fig. 4.3C). Samples treated with HCA and glucose had lower MBC:MBN ratios than the control, except for WS+P, although the differences between the treatments were not significant (Fig. 4.3F).

#### 4.3.4 The effect of HCA, glucose, and P addition on the dynamics $^{13}\text{C}$ in soil microbial biomass



**Figure 4.13** Dynamics of the content of  $^{13}\text{C}$  in microbial biomass and the  $\delta^{13}\text{C}$ -MBC recovery during the incubation experiment.

Addition of HCA as a single factor had a significant effect on the uptake of  $^{13}\text{C}$  by microorganisms, but not the interaction between P and HCA (Table 4.4). The content of  $^{13}\text{C}$  in microorganisms ( $\delta^{13}\text{C}$ -MBC) generally increased during the incubation (Fig. 4.4A). Compared with glucose addition only, co-application with P increased the  $\delta^{13}\text{C}$ -MBC, while HCA decreased the value compared to the control, even when combined with P application (Fig. 4.4A).

**Table 4.7** Summary results of repeated measures ANOVA for the main effects of high carbon amendment (HCA: WS or SD), phosphorus (P), and their interactions on the  $^{13}\text{C}$ -glucose uptaken by microbial biomass and  $\delta^{13}\text{C}$ -MBC recovery.

Interaction	$\delta^{13}\text{C}$ -MBC (‰)			$\delta^{13}\text{C}$ -MBC recovery (%)		
	<i>P</i>	<i>F</i>	<i>Eta</i>	<i>P</i>	<i>F</i>	<i>Eta</i>
P	0.630	0.254	0.035	0.396	0.803	0.091
WS	<b>&lt;0.05</b>	40.089	0.909	0.420	0.770	0.133
WS * P	0.968	0.002	0.001	0.852	0.039	0.008
SD	<b>&lt;0.05</b>	9.372	0.652	0.141	3.063	0.380
SD*P	0.261	1.601	0.243	<b>&lt;0.05</b>	7.475	0.599

WS: wheat straw; SD: sawdust; MBC: microbial biomass carbon; #Eta = Partial eta squared is a measure for the effect size of a variable, calculated as the ratio of variance associated with an effect, plus that effect and its associated error variance.

The  $^{13}\text{C}$  recovery in MBC was significantly affected by the interaction of sawdust and P addition (Table 4.4). The  $^{13}\text{C}$  recovery in MBC increased with incubation time and was highest in SD+G+P throughout the experiment (Fig. 4.4B). The  $^{13}\text{C}$  recovery in MBC was in the order of 2.8% (SD+G+P) > 2.3% (G) > 2.2% (G+P) > 1.9% (WS+G+P) > 1.9% (W+G) > 1.8% (SD+G) at the end of the experiment (Fig. 4.4B).

## 4.4 Discussion

### 4.4.1 Combined effect of HCA, glucose, and P on GHG emissions

To date, numerous studies have concluded that even minor changes in SOM content and quality can have a profound effect on microbial biomass and activity, which is, at least partially, reflected in soil  $\text{CO}_2$  emission (Chaker et al., 2019; Cleveland and Liptzin, 2007; Jäger et al., 2011; Johnston et al., 2009). In agreement with the results of previous studies (Curtin et al., 1998; Li et al., 2021a; Wu et al., 2013), we found that samples with HCA addition showed higher  $\text{CO}_2$  emissions compared with the control (Fig. 4.1D). There was a substantial increase in  $\text{CO}_2$  flux especially during the first week of the experiment, indicating that C and nutrient limitation of microbial activity was alleviated after the addition of C, N, and P substrates (Chen

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et al., 2014). The increase in soil respiration after HCA addition can be considered as an indicator of increasing soil microbial activity (Johnston et al., 2009), implying that addition of HCA to soil provides an important exogenous C source for the microbial metabolism (Kamau et al., 2021), and that labile C is the critical factor that stimulates microbial activity, which in turn accelerates the decomposition of recalcitrant substrates. With respect to abiotic effects, the application of HCA can increase SOM degradation and soil respiration through increasing WHC and soil porosity, i.e., soil aeration (Barzegar et al., 2002; Wang and Feng, 2010). However, we did not observe a significant effect of HCA addition on WHC and soil porosity in our experiment.

The effects of HCA are mainly determined by the degradability and the duration of the supply of labile C to soil microorganisms (Li et al., 2021b; Xia et al., 2014). In our experiments, soil amended with wheat straw and glucose (with/without P) had higher CO<sub>2</sub> emission than that amended with sawdust only (C:N ratio up to 400) during the first week of incubation (Fig. 4.1A). The C:N ratio and recalcitrance of HCA added to the soil are important factors controlling the SOM decomposition processes (Nicolardot et al., 2001). During the decomposition of HCA with low C:N ratio, the available C and N can be more rapidly utilized by soil microorganisms compared to decomposition of HCA with high C:N ratio, which is beneficial for the development of the microbial community and accelerates the decomposition of SOM in the initial period of experiment (Chen et al., 2014). As an easily decomposable HCA despite its infinitely high C:N ratio, glucose can provide energy to microorganisms and stimulate decomposition of more recalcitrant HCA and SOM (Blagodatskaya et al., 2007). Due to the high availability of glucose, a larger amount of CO<sub>2</sub> was released from the glucose-amended treatments in the initial phase, temporarily increasing CO<sub>2</sub> emission (Fig. 4.1A) (Kuz'yakov and Demin, 1998). In contradiction to our first hypothesis that a stimulation of HCA decomposition would result in significantly increased soil CO<sub>2</sub> emissions, we found no significant effect of glucose, P, HCA, and their interaction on stimulating CO<sub>2</sub> emission over the whole duration of the experiment (Table 4.1, Fig. 4.1D). This observation suggests effective sequestration of carbon in microbial biomass through efficient recycling also of supposedly 'labile' carbon, reflected in the constantly increasing <sup>13</sup>C content of microbial biomass over the whole duration of the experiment (Fig. 4.4A). The lack of a significant difference in CO<sub>2</sub> emission between samples with and without P addition further supports the assumption that the alleviation of nutrient limitation of microbial growth and activity is not necessarily translated to higher respiration rates but leads to more carbon-use efficient build-up of soil microbial biomass.

Soil CH<sub>4</sub> emission is the net result of the activity of methanogens and methanotrophs (Conrad, 2009; Dutaur and Verchot, 2007). In our experiment, HCA application turned the soil from a net CH<sub>4</sub> sink (control) to a small net CH<sub>4</sub> source. The incorporation of HCA could have, to some

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extent, stimulated the activity of methanogens by providing them with a readily available C source (Khosa et al., 2010; Zou et al., 2005). In addition, an increase in microbial O<sub>2</sub> consumption in the soil induced by HCA degradation might have created anaerobic conditions suitable for the formation of CH<sub>4</sub> (Conrad, 1996). Our data also showed that the CH<sub>4</sub> flux was reduced at lower temperature. The reason could be that methanogens with a Q<sub>10</sub> around 1.5-28 are more sensitive to a decrease in temperature than methanotrophs, which are less affected by temperature changes, leading to a relatively higher CH<sub>4</sub> oxidation compared to CH<sub>4</sub> formation at lower temperature (Ding and Cai, 2003).

In line with our second hypothesis, soil treated with HCA emitted only marginal amounts of N<sub>2</sub>O during the incubation experiment, except for WS, which had comparable N<sub>2</sub>O emission like the control (Fig. 4.1F). Typically, crop residues stimulate N<sub>2</sub>O emission from soil as reported by Li et al. (2021b). However, an increased N retention by microbial growth after HCA application can also inhibit N<sub>2</sub>O emissions due to limitation of N availability (Ma et al., 2007; Ma et al., 2009; Millar and Baggs, 2005). Carbon-rich wheat straw or sawdust can stimulate microbial N immobilization (Toma and Hatano, 2007; Vigil and Kissel, 1991), thereby reducing the amount of available mineral N for nitrification and denitrification processes. Second, soluble C released during HCA decomposition could increase microbial respiration and decrease soil redox potential, which could promote the reduction of N<sub>2</sub>O to N<sub>2</sub> during denitrification (Millar and Baggs, 2005).

Previous research showed that P fertilization has the potential to increase N immobilization and mitigate N<sub>2</sub>O emission (Sundareshwar et al., 2003). In contrast, Mori et al. (2010) came to the opposite conclusion that N<sub>2</sub>O emission can increase after P addition in a tropical evergreen broad-leaved forest through affecting the N supply to nitrifying and denitrifying microorganisms. O'Neill et al. (2020) found that the addition of P had no significant effect on N<sub>2</sub>O emissions in C-limited soils, while N<sub>2</sub>O emissions were higher in soils with low P content than in soils with high P content after C addition.

Similarly, in our experiment, application of sawdust only reduced N<sub>2</sub>O emission significantly, however, there was no difference in the average N<sub>2</sub>O emission between SD or SD+P. Hence, the differences between our results and those of other studies suggest that the effect of P on N<sub>2</sub>O can be affected by both the initial P content of the soil and soil C availability.

#### **4.4.2 Combined effect of HCA, glucose, and P on soil nutrients**

Amending soils with HCA to improve soil fertility is a common practice worldwide that directly and/or indirectly affects soil N retention through biotic and abiotic factors such as soil temperature, C and P availability, soil microbial activity, and soil aggregate formation (Blanco-Canqui and Lal, 2007; Jin et al., 2020). Our results show that HCA addition increased soil DOC concentration even after eight months of incubation, implying that the effects of HCA

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decomposition can last for a longer period of time. HCA addition decreased the  $\text{NO}_3^-$  content in our experiment (Fig. 4.2B). Previous research has shown that soil microorganisms are stimulated to mine available N from the soil after addition of labile C to maintain the narrow C:N ratio of around 6-8, implying that increasing soil microbial biomass is an effective way to improve soil N retention (Cleveland and Liptzin, 2007). In line with our second hypothesis, the application of HCA clearly decreased the  $\text{NO}_3^-$  leaching potential by stimulating microbial N retention. Our results also indicate that sawdust had a high potential for soil N retention in the longer term since the C release from sawdust can persist for a much longer time than from more readily degradable substrates. In line with our first hypothesis, the addition of glucose significantly enhanced the potential of sawdust to increase soil N retention. This is because glucose as a readily available C source can be directly utilized by microorganisms and promote microbial growth, thereby potentially also accelerating the decomposition of recalcitrant substrates (Mehnaz et al., 2019) and shortening the lag period until the soil microorganisms have adapted to a specific new HCA type (i.e., bridging the gap between short-term and long-term N immobilization) (Dunn et al., 2006).

Soil available P is also an important factor affecting HCA-mediated N retention. In our study, the experimental soil was collected from a recultivation area characterized by a low level of available P, high pH (7.5) and a high calcium carbonate ( $\text{CaCO}_3$ ) content (Li et al., 2021a). Therefore, it was obvious to anticipate a P limitation of microbial activity in our experiment due to potential P immobilization in the form of apatite at this pH. However, in our experiment, soil  $\text{NO}_3^-$  content responded similarly to the addition of wheat straw and sawdust only as well as to the combined addition of P and HCA (Fig. 4.2E), despite the tendency for lower nitrate levels in the P-amended WS, SD and G treatments, but which was not significant ( $P > 0.05$ ). Therefore, we can assume that the C:N:P balance required for stimulating microbial activity related to N retention was also sufficiently established in the non-P-amended soil.

As a C source that can be rapidly decomposed and utilized by microorganisms, glucose has been widely used in various culture experiments (Hill et al., 2008; Xu et al., 2019). In addition, it can also stimulate microbial growth provided there is sufficient N (Elgharably and Marschner, 2011). Vinten et al. (2002) found higher immobilization of N by combined application of wheat straw and glucose. Our results also showed a stimulating effect of glucose in combination with wheat straw and sawdust on the  $\text{NO}_3^-$  content during the experiment, which was significant in the case of sawdust (Fig. 4.2B, E). In contrast, glucose addition alone did not lead to a significant reduction of soil  $\text{NO}_3^-$ , suggesting that the effect of exogenous C on soil N retention is depending on a delicate balance of readily available and more recalcitrant carbon. Contrary to our expectation, and as already depicted in the previous paragraph, additional P supply did not have any further significant effect on  $\text{NO}_3^-$  retention (Fig. 4.2E), indicating that the soil P supply was sufficient for microbial activity also without exogenous P source.



#### 4.4.3 Combined effect of HCA, glucose, and P on soil microbial biomass

Pearson correlation analysis showed that the microbial biomass content was inversely correlated with the residual  $\text{NO}_3^-$  content of the soil (Table 4.5), which adds further evidence for the uptake and immobilization of N by soil microorganisms provided there is sufficient C available. High carbon amendments decomposition provides the exogenous C source for the growth and activity of soil microorganisms (Cochran et al., 1988). In our experiment, soil MBC gradually increased throughout the incubation period, suggesting that nutrients were still present in the soil at the end of the incubation experiment that could be used by the microorganisms. In a practical sense, this implies that the stimulatory effect of HCA addition on N retention may last longer than anticipated and that this prolonged N retention has to be taken into account in the following year of the crop rotation.

**Table 4.8** Pearson correlation coefficients between soil nitrate ( $\text{NO}_3^-$ ), extractable P ( $P_{\text{ex}}$  = extracted with 0.01 M  $\text{CaCl}_2$ ), dissolved organic carbon (DOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and MBC:MBN during the experiment.

	Nitrate	$P_{\text{ex}}$	DOC	MBC	MBN	MBC:MBN
Nitrate	1	0.008	-0.381**	-0.249**	-0.175**	0.041
$P_{\text{ex}}$	0.008	1	0.050	-0.244**	-0.030*	0.040
DOC	-0.381**	0.050	1	0.466**	0.046*	0.161**
MBC	-0.249**	0.244**	0.466**	1	0.451**	-0.010
MBN	-0.175**	-0.030	-0.046	0.451**	1	-0.409**
MBC:MBN	0.041	0.040	0.161**	-0.010	-0.409**	1

Compared with the control, the addition of HCA and glucose led to higher MBN content and thus to an increased immobilization of soil mineral N. In our experiment, the MBN content of samples amended with HCA and the control without HCA was similar ( $P > 0.05$ ). In contrast, co-application of HCA and glucose resulted in a significant increase in soil MBN content (Fig. 4.3E). The reason for this may very likely be the fact that 1) the C:N:P stoichiometric ratio of microbial biomass is quite narrow, and soil microorganisms therefore were forced to mine N and P by degrading humus for growth and activity in the C-enriched soil (Cleveland and Liptzin, 2007); 2) glucose as an additional fast C resource increased microbial activity, which in turn accelerated the decomposition of HCA and thus increased the MBN content.

The MBC:MBN ratio partially reflects the abundance of bacteria and fungi in the soil because, as fungi generally have a C:N ratio twice or even higher than bacteria (Qiao et al. 2021). Previous studies showed that MBC:MBN increased or decreased after C addition (Li et al., 2021a; Singh et al., 2016; Song et al., 2018). We observed a tendency to lower MBC:MBN values compared to the control and the P only treatment, suggesting that the contribution of

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bacteria to the total soil microbial biomass increased due to HCA addition. However, due to the high variability between replicates, this trend was not significant, also not in combination with glucose or P addition. We conclude that for variables that are calculated as the quotient of two measured variables, the number of replicates in our study was insufficient.

#### **4.4.4 Combined effect of HCA, glucose, and P on $\delta^{13}\text{C}$ incorporation in microbial biomass**

$^{13}\text{C}$ -labelled OM is generally used to assess the preference of soil organisms for different C substrates at different soil conditions, and is helpful to understand soil C cycling in the biosphere and its interaction with soil N dynamics (Kögel-Knabner, 1997). Several studies have been conducted to investigate the effect of C addition on soil microbial dynamics and on soil N losses; however, most of these studies only focused on glucose but ignored its interaction with HCA (Chenu et al., 2001; Dungait et al., 2011). A continuous input of glucose resembles the slow decomposition of residues. A few studies have examined the effect of continuous glucose input on OM decomposition (Yevdokimov et al., 2006), leading to an increase in microbial activity and biomass as well as SOM turnover. However, few studies applied glucose only initially, and continuously observed the effect for a longer time. In contrast to previous research (De Troyer et al., 2011), we found that the  $\delta^{13}\text{C}$ -MBC and the recovery of  $^{13}\text{C}$  in microbial biomass continued to increase during the experiment (Fig. 4.4). Furthermore, the MBC content was higher, while the  $\delta^{13}\text{C}$ -MBC was lower, in soil amended with HCA and glucose than in soil amended with glucose only (Fig. 4.3D, 4.4A), suggesting that the available C released by HCA decomposition became a significant source of MBC and thereby diluted the  $^{13}\text{C}$ -labelled C source (glucose) in the soil.

Mehnaz et al. (2019) showed that the P fertilization can increase the recovery of glucose-C because a lack of P can limit microbial activity as it is required for the synthesis of genetic and cellular components. Similar to their findings, our results showed that P addition significantly increased the recovery of  $^{13}\text{C}$  in soil microbial biomass in the sawdust- and glucose-treated samples, and this effect became more pronounced with increasing incubation time. In contrast, P addition had no significant effect on  $^{13}\text{C}$  recovery in soil amended with wheat straw and glucose. A potential explanation for this difference between wheat straw and sawdust is that the decomposition of sawdust required additional P. These findings from different experiments suggest that the efficiency of C utilization by soil microorganisms depends on the C:N ratio and decomposability of the substrate, and less on the soil P availability above a critical threshold.

#### **4.5 Conclusions**

This study assessed whether combined application of HCA with glucose and P can improve the ability of HCA to retain N in soil. Our results demonstrated that particularly the combination of glucose with sawdust, and to a lesser extent with wheat straw, stimulated microbial growth

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and led to significantly lower soil  $\text{NO}_3^-$  content at the end of the eight-month incubation experiment. This combination of readily available and more recalcitrant C was also associated with a significantly lower  $\text{N}_2\text{O}$  emission than the non-amended control and the wheat straw only treatment. Our work showed that also more recalcitrant HCA types, such as sawdust, may reduce N losses from post-harvest fields if sufficient labile C and P are available. However, since we used a strongly C-, N- and P-limited recultivation soil in our study, the general applicability of the results has to be further evaluated with various soils under field conditions to strengthen the evidence.

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## Chapter 5

### **Effects of compost incorporation on greenhouse gas emissions, N retention, and crop yield at two different P levels: a lysimeter experiment**

Based on:

Li Z, Reichel R, Wissel H, Cao X, Pütz T, Küpper W, Meulendick P, Zhao K, Endenich M, Brüggemann N. Effects of compost incorporation on greenhouse gas emissions, N retention, and crop yield at two different P levels: a lysimeter experiment. *Manuscript in preparation*.

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## 5.1 Introduction

With the increase of the global population, the food demand is increasing at the same time, prompting farmers to apply increasing rates of artificial fertilizers to maintain or increase agricultural productivity. However, mineral fertilization beyond plant requirements will inevitably lead to an imbalance of nutrients in the soil, a loss of humus and biological functions to regulate nutrient availability, leaching of excess nutrients causing surface water eutrophication and the contamination of groundwater bodies (Ersahin, 2001; Kleinman et al., 2011), and increased emission of N trace gases, such as  $\text{N}_2\text{O}$ ,  $\text{NO}$ , and  $\text{NH}_3$ , suggesting that mineral fertilization alone undoubtedly leads to large environmental problems and puts soil resilience to climate change at risk (Lal, 2006; Tilman et al., 2011). Furthermore, soil microorganisms are often sensitive to changes in available nutrient sources, especially under conditions of N excess in the soil (Cleveland and Liptzin, 2007). Fertilizer applied in excess of the amount taken up by microorganisms and plants accumulates in the soil and affects the development of the microbial community (Liu et al., 2012). Moreover, the excess fertilizer N stimulates the activity of nitrifying and denitrifying microorganisms and further exacerbates the emission of  $\text{N}_2\text{O}$  (Li et al., 2021c). Besides  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  are important GHGs. The GWP of these gases are approximately 25 and 298 times greater, respectively, than that of  $\text{CO}_2$  over a time span of 100 years (IPCC, 2021). Therefore, conventional agriculture must find ways to increase sustainability, optimize nutrient efficiency, and protect the environment while at least maintaining crop yields as the basic criteria of a bio-based economy.

There is increasing recognition that incorporating soil amendments with high organic C content can keep more fertilizer N in the soil and protect it from leaching (Gheysari et al., 2009; Wang et al., 2019b). The use of organic fertilizers in appropriate form and quantity holds great potential to increase soil nutrient storage and crop yields while mitigating global warming through increased SOC storage (Agegnehu et al., 2016). Compost is a good source of nutrients, and increases SOM content at the same time, which has a positive effect on soil structure, WHC, nutrient retention (Hargreaves et al., 2008; Mohanty et al., 2013), and crop productivity (Erhart et al., 2005; Tejada and Gonzalez, 2003), and suppresses many diseases caused by soil-borne plant pathogens (Cotxarrera et al., 2002). However, improper use of compost might cause serious environmental pollution. For example, various gases, particularly  $\text{NH}_3$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ , released during composting can affect air quality; in addition, water quality can be affected by the loss of mineral N, i.e., by  $\text{NO}_3^-$  leaching (Peigné and Girardin, 2004). As a result, the positive effect of compost application on sustainable agricultural development might be offset by inappropriate application.

Phosphorus (P) is a critical component of RNA, DNA, phospholipids, and ATP, and can be stored in plants in larger quantities as phytate. The availability of P in the soil affects

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biogeochemical processes related to synthesis and decomposition of SOM (Smeck, 1985). The utilization efficiency of N by microorganisms and plants is strongly affected by the availability of P (Li et al., 2021a; Mehnaz and Dijkstra, 2016; Mori et al., 2010). However, there is no consensus on how different soil P levels affect N losses. For instance, P fertilization increased N immobilization and mitigated N<sub>2</sub>O emissions in a coastal ecosystem in South Carolina, USA (Sundareshwar et al., 2003). Hall and Matson (1999) reported greater N<sub>2</sub>O emissions from P-limited than N-saturated tropical forest soils. Conversely, P addition alleviated microbial P limitation and increased soil N<sub>2</sub>O emission in a tropical evergreen broad-leaved forest as a result of N supply to nitrifying and denitrifying microorganisms (Mori et al., 2010). Tate and Salcedo (1988) showed that P availability controlled SOM accumulation and re-mineralization. Consequently, P availability can affect soil C and N cycling, which makes an influence on nutrient storage and release from compost likely.

For the research of interaction between soil nutrients and crop yield, the traditional field experiment is the most common method. In the field, however, it is difficult to monitor changes in nutrient losses by leaching and runoff in real time on a long-term and frequent basis (Pütz et al., 2018). The laboratory incubation experiment is another research method besides field experiments, but under more controlled conditions. However, the sample size of such incubation experiments is usually small and the conditions may differ from the natural environment (Leng et al., 2019). As a system for measuring C and N balance and quantifying losses with leachate and in gaseous form, lysimeters provide a link between laboratory and field conditions, combining their strengths and avoiding deficiencies. It is therefore a valuable experimental tool for studying biogeochemical processes in soils under controlled, yet realistic conditions, and for measuring complex interactions between soil, plants, water, nutrients and chemicals (Kay et al., 2004; Pütz et al., 2018).

The objective of this study was to quantify GHG emissions, soil nutrient retention and leaching, and aboveground biomass and crop yield at the same common mineral N fertilizer level, but different compost and P fertilizer levels, in a two-year outdoor lysimeter experiment with soil low in SOC and nutrient content. Four hypotheses guided our experiment: (1) compost application will not lead to a significant increase in GHG emissions due to efficient (re)cycling of C, N, and P in the soil-plant system; (2) additional mineral P fertilization stimulates substrate mineralization and thus releases additional N, which further increases plant growth and yield; (3) combined application of compost and mineral N and P fertilizer will not lead to increased N and P leaching due to efficient microbial and plant immobilization of nutrients; (4) increasing compost application rates will increasingly enhance plant growth and yield compared to the control without compost due to enhanced supply of nutrients and water to the plants.

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## 5.2 Materials and methods

### 5.2.1 Experimental site and design

The lysimeters with undisturbed soil cores were sampled in May 2019, using stainless steel cylinders of 0.5 or 1.0 m<sup>2</sup> surface area and 1.15 m depth (Fig. S5.1), from recultivated fields at the Inden lignite mine (50.88° N, 6.37° E) close to Jülich (Nordrhein-Westfalen, Germany). In this area, RWE Power AG (Essen, Germany) removes former agricultural soil and subjacent bedrock layers before lignite extraction. During this process, loess loam of former topsoil and loess parent material are mixed and deposited at the backside of the mine. Three months after soil settling, redeposited soil substrate is leveled and planted with alfalfa for three to four years. During this phase, the fields receive an initial fertilization (N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, each 30 kg ha<sup>-1</sup>), but no biocide treatments. The basic soil properties (0-30 cm) were as follows: pH: 7.54 (CaCl<sub>2</sub>), NH<sub>4</sub><sup>+</sup>-N: 0.14 mg kg<sup>-1</sup>, NO<sub>3</sub><sup>-</sup>-N: 0.46 mg kg<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub>: 16.50 mg kg<sup>-1</sup>, SOC: 3.5 g kg<sup>-1</sup>, and TN: 0.5 g kg<sup>-1</sup>.

The lysimeter experiment was conducted at Forschungszentrum Jülich GmbH (Germany) with winter wheat (*Triticum aestivum*) from October 29, 2019 (sowing date) to July 28, 2020 (harvest), and winter barley (*Hordeum vulgare*) from October 22, 2020 to August 2, 2021, respectively.

We used a randomized block design with five treatments, comprising soil with 0, 40 (area-typical), and 80 t green-waste compost ha<sup>-1</sup>, and two P fertilization levels (area-typical and twice the amount; see Table S5.1 for the specific amounts applied in each season): (1) P1: soil with area-typical P fertilization (control); (2) P2: soil with twice the typical P fertilization; (4) CP1: soil with area-typical compost + typical P fertilization; (4) CP2: soil with area-typical compost + twice the typical P fertilization; (5) CCP1: double amount of area-typical compost + typical P fertilization. Each treatment has 4 replicates.

**Table 5.9** The basic properties of the green-cutting compost

DOC	DN	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> <sup>+</sup> -N	P-CAL	OC	TN	C/N
mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	
3368.3	689.8	7.6	497.1	1584	211	18	11.7

DOC: dissolved organic carbon; DN: dissolved total nitrogen; P-CAL: plant-available P; OC: organic carbon; TN: total Nitrogen

The green-waste compost contained 21.1% C, 1.8% N, and 0.16% extract plant-available P (P-CAL) (Table 5.1), was applied once before ploughing and winter wheat sowing. N delivery from decomposition of remaining alfalfa material was estimated to be equivalent to 20 kg N ha<sup>-1</sup>. The lysimeters were conventionally managed according to area-typical plant-fertilization and

protection practice in accordance with RWE Power AG and the German Fertilizer Ordinance (Kuhn, 2017). We used the following fertilizers: YaraMila Universal (6.3%  $\text{NO}_3^-$ -N, 8.7%  $\text{NH}_4^+$ -N, 15%  $\text{P}_2\text{O}_5$ , 5%  $\text{K}_2\text{O}$ , 3.7%  $\text{SO}_3$ ; YARA GmbH & Co. KG, Dülmen, Germany), CAN (13.5%  $\text{NO}_3^-$ -N, 13.5%  $\text{NH}_4^+$ -N; Raiffeisen Waren-Zentrale Rhein-Main eG, Köln, Germany), and triple superphosphate (46%  $\text{P}_2\text{O}_5$ ; Raiffeisen Waren-Zentrale Rhein-Main eG, Köln, Germany). Details about the fertilizer timing and amount are provided in Table S5.1.

## 5.2.2 Measurement of GHG emissions

Greenhouse gas emissions in the lysimeter experiment were measured with a Fourier Transform Infrared (FTIR) analyzer (DX4015, Gaset Technologies Oy, Helsinki, Finland) with the closed dynamic chamber technique. For each lysimeter, three PVC sewage pipes with end plugs (5 cm diameter x 25 cm length) were used as chambers and randomly pushed into the soil to a final soil depth of 15 cm to improve the coverage of spatial heterogeneity. Immediately before each measurement, gas-tight caps with inlet and outlet port were screwed on the PVC chambers and connected in parallel to the FTIR analyzer via ¼ inch PTFE tubing over two cross junctions. For each lysimeter, a continuous GHG measurement was conducted for 6 minutes, and mean concentration readings were recorded in 20-second intervals. During the two-year experiment, the fluxes of GHG were measured once every two weeks between harvest and the first fertilization in spring, and once per week in the growing season (period between first fertilization and harvest). The frequency of GHG emission measurements was further increased to three times per week after fertilization events. GHG emission was assumed greater or less than zero if there was a significant relationship between concentration change in the chamber headspace and time at  $R^2 > 0.81$ . The following formula was used to calculate the  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  flux:

$$F = \frac{\frac{\Delta C}{\Delta t} \times V \times T_0 \times M}{A \times T_a \times V_m}$$

where  $F$  represents the gas emission flux;  $\Delta C/\Delta t$  represents the change of gas concentration in ppmv for  $\text{CO}_2$  and ppbv for  $\text{CH}_4$  and  $\text{N}_2\text{O}$ ;  $V$  represents the headspace volume in liter;  $M$  represents the molar mass of N in  $\text{N}_2\text{O}$  or C in  $\text{CO}_2$  or  $\text{CH}_4$ , respectively;  $A$  represents the combined surface area of the chambers of one lysimeter;  $V_m$  represents the molar volume of ideal gases ( $22.414 \text{ L mol}^{-1}$ ) at  $0^\circ\text{C}$  and  $101.325 \text{ kPa}$ , corrected for the gas sample temperature using  $T_0$  ( $273.15 \text{ K}$ ) and  $T_a$  (air temperature in  $\text{K}$ ) (Reichel et al., 2018). Cumulative emissions were calculated as the sum of the areas of the trapezoids formed by the two respective flux rate values of adjacent measurement dates and the time difference as the base (Xia et al., 2014).



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### 5.2.3 Soil extraction and analysis

A customized stainless steel soil auger with a diameter of 8 mm was used for soil sampling to minimize the disturbance of the lysimeters. Soil samples were collected from the topsoil (0-25 cm) at four dates, covering four seasons, which were 1: autumn/winter; 2: early spring (before fertilization); 3: early summer (crop maturing); 4: midsummer (after harvest). Nine soil cores were randomly taken from each lysimeter, mixed, and sieved to 2 mm to get a composite soil sample free of visible plant material and stones. Fresh soil equivalent to 10 g dry soil was mixed with 40 mL 0.01 M  $\text{CaCl}_2$  in 50-mL Falcon tubes (VWR International, Darmstadt, Germany), shaken horizontally at a speed of 200 turns  $\text{min}^{-1}$  for 60 min, centrifuged at  $690 \times g$  for 20 min, and filtered through sterile syringe filters (25 mm, 0.45  $\mu\text{m}$  polypropylene membrane syringe filter, VWR Europe) to measure soil  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and DOC. Mineral N ( $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) was measured with ion chromatography (DX-500, Dionex). Dissolved organic C was measured with a total organic C (TOC) analyzer (TOC-VcPH + TNM-1 + ASI-V, Shimadzu). Two gram of air-dry, sieved soil was mixed with 0.05 M calcium lactate / calcium acetate (CAL) solution (pH 4.5) at a ratio of 1:20 (m/v), then shaken at 200 turns  $\text{min}^{-1}$  for 90 min to extract P-CAL. The following sample preparation was identical to the extraction of mineral N. P-CAL was determined by inductively coupled plasma optical emission spectroscopy (iCA 7600, Thermo Fisher Scientific). Before measurement, all samples were frozen at  $-20^\circ\text{C}$ .

### 5.2.4 Measurement of nutrient leaching

Leachate was checked weekly and sampled only when more than 5 cm had accumulated in the reservoir below the lysimeter. The leachate amount was determined by the mass of water pumped out of the collection tank of each lysimeter. One hundred mL leachate was filtered through 0.45  $\mu\text{m}$  PP-membrane filters (disc size 25 mm; Macherey-Nagel, Düren, Germany) for the analysis of DOC,  $\text{NH}_4^+$ -N, and  $\text{NO}_3^-$ -N. Leachate samples were frozen at  $-20^\circ\text{C}$  immediately after filtration, and measured as described above.

### 5.2.5 Determination of crop parameters

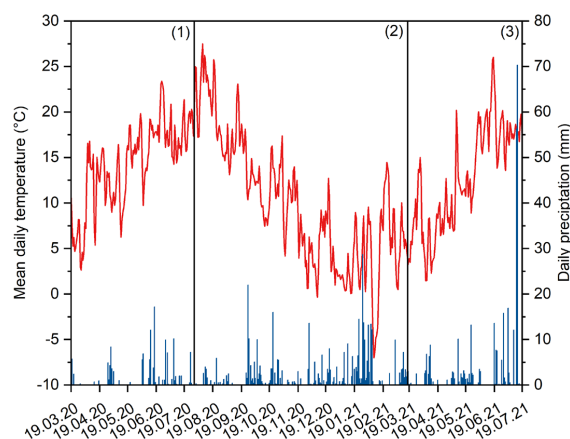
At harvest, whole wheat and barley plants were cut 1 cm above the ground, and air-dried to determine the total above ground biomass. Shoots were separated from ears and oven-dried at  $60^\circ\text{C}$  before threshing. Threshing was performed with a laboratory thresher (Haldrup LT-20, Inotec Engineering GmbH, Ilshofen, Germany). About 1-2 handful of ears were placed in the hopper of the machine (set at level 5). After turning off the engine, the fan was turned on to level 10 to separate the awns and husks from the grains. Hereafter, the total mass of the grains was determined. Moisture of the grains was determined with a humidity meter (Pfeuffer HE lite, Pfeuffer GmbH, Kitzingen, Germany).

## 5.2.6 Statistical analysis

Repeated-measures ANOVA was performed to test the effects of compost, P fertilizer level, and their interactions on GHG emissions, soil nutrient retention, and leaching. Two-way ANOVA was used to test the effect of the compost, fertilizer P-level, and their interactions on total crop aboveground biomass and grain yield. One-way ANOVA was conducted to check the difference in GHG emissions between the treatments. The differences were considered significant when  $P < 0.05$ . All statistical analyses were performed using SPSS Statistics 21.0 (IBM Deutschland GmbH, Ehningen, Germany).

## 5.3 Results

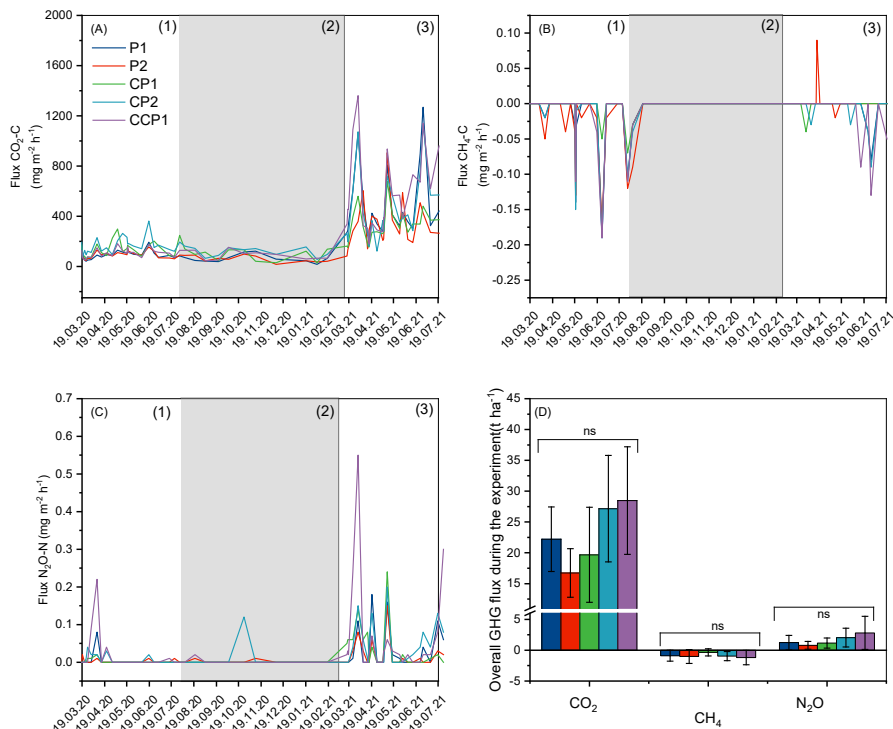
### 5.3.1 Meteorological data



**Figure 5.14** Dynamics of daily mean temperature and total precipitation during the two growing seasons in lysimeter experiment. (1), (2), and (3) indicates the wheat growing season (March 19, 2020 – July 28, 2020), non-growing season (July 29, 2020 – March 16, 2021), and barley growing season (March 17, 2021 – July 27, 2021), respectively.

The maximum temperature was 39.0 °C on July 25, 2019, and the minimum temperature was -10.6°C on February 10, 2021. The daily temperature changes were similar between the two growing seasons (winter wheat growing season: March 19, 2020 – July 28, 2020; winter barley growing season: March 17, 2021 – July 27, 2021), and the mean air temperature in the wheat and barley growing seasons was 14.2 °C and 13.0 °C, respectively. The maximum daily precipitation in the wheat and barley growing season was 17.2 and 70.3 mm, respectively. The total precipitation amount in the wheat and barley growing season was 138.7 mm and 326.1 mm, respectively (Fig. 5.1).

### 5.3.2 Soil GHG fluxes and emissions



**Figure 5.15** Seasonal variation of CO<sub>2</sub>-C (A), CH<sub>4</sub>-C (B), and N<sub>2</sub>O-N (C) flux during the wheat–barley rotation period from 2020 to 2021, and the overall GHG flux during the experiment (D). P1: low phosphorus (Control); P2: high phosphorus; CP1: low compost + low phosphorus; CP2: low compost + high phosphorus; CCP1: high compost + low phosphorus. (1), (2), and (3) indicate the wheat growing season (March 19, 2020 – July 28, 2020), non-growing season (July 29, 2020 – March 16, 2021), and barley growing season (March 17, 2021 – July 27, 2021), respectively.

Repeated-measures ANOVA revealed that the CO<sub>2</sub> flux was significantly increased by the compost addition in the wheat growing season, while in the barley growing season a significant interactive effect between compost addition and P fertilization on CO<sub>2</sub> emission was found (Table 5.2). During the entire wheat–barley crop rotation, the CO<sub>2</sub> flux ranged between 16.59 and 1792.43 mg m<sup>-2</sup> h<sup>-1</sup>, both found in the P1 (Fig. 5.2A(1)). Compared with P1, the CCP1 and CP1 treatment had a 12% and 33% higher average CO<sub>2</sub> flux in the winter wheat growing season, respectively (Fig. 5.2A(1)), while the extra P fertilization in the P2 treatment had no significant effect on the average CO<sub>2</sub> flux compared with P1. However, compost and extra P fertilization (CP2) increased the average CO<sub>2</sub> flux by 43% compared to P2 in the winter wheat growing

season (Fig. 5.2A(1)). Soil CO<sub>2</sub> flux was 393% (P1), 243% (P2), 129% (CP1), 172% (CP2), and 432% (CCP1) higher in the winter barley growing season than in the winter wheat growing season. In the CCP1 treatment, the CO<sub>2</sub> flux was 19% higher compared to P1 in the barley growing season, while all other treatments showed a reducing effect on soil CO<sub>2</sub> flux compared to P1 (Fig. 5.2A(3)). However, there was no significant difference in the cumulative CO<sub>2</sub> emissions between the treatments, neither in the wheat nor in the barley growing season (Table S5.3).

**Table 5.10** Results of repeated measures ANOVA for the main effects of compost (C), phosphorus (P), and their interactions on fluxes of CO<sub>2</sub>-C, CH<sub>4</sub>-C, and N<sub>2</sub>O-N in wheat and barley growing season.

Period	GHG	CO <sub>2</sub> -C			CH <sub>4</sub> -C			N <sub>2</sub> O-N		
		P	F	Eta	P	F	Eta	P	F	Eta
Wheat season	P	0.140	2.422	0.139	0.189	1.880	0.105	0.816	0.056	0.003
	C	<b>&lt;0.01</b>	6.670	0.471	0.263	1.454	0.154	0.235	1.588	0.166
	P * C	0.145	2.359	0.136	0.477	0.530	0.032	0.598	0.290	0.018
Barley season	P	0.891	0.020	0.001	0.074	0.789	0.05	0.721	0.132	0.008
	C	0.128	2.346	0.227	1.404	0.274	0.149	0.134	2.285	0.222
	P * C	<b>&lt;0.05</b>	5.058	0.240	3.046	0.100	0.160	0.415	0.529	0.025

Eta: Partial Eta Squared

Repeated-measures ANOVA showed that compost addition and P fertilization caused no significant effect on CH<sub>4</sub> flux in any of the respective treatments of the lysimeter experiment (Table 5.2). The majority of the CH<sub>4</sub> uptake occurred in the wheat growing season (Fig. 5.2B). The maximum soil CH<sub>4</sub> uptake flux in the wheat and barley growing seasons was -0.19 mg m<sup>-2</sup> h<sup>-1</sup> and -0.13 mg m<sup>-2</sup> h<sup>-1</sup>, respectively (Fig. 5.2B), in the CCP1. In the wheat growing season, the CCP1 and CP1 treatment had a higher CH<sub>4</sub> uptake flux compared with P1, whereas the P2 treatment had a 19% lower CH<sub>4</sub> uptake flux (Fig. 5.2B(1)). A similar pattern was found in the barley growing season (Fig. 5.2B(3)). However, there were no significant differences in the cumulative CH<sub>4</sub> emission between the treatments in the wheat and barley growing season (Table S5.2).

The majority of the N<sub>2</sub>O emissions occurred in the barley growing season (Fig. 5.2C). The maximum N<sub>2</sub>O flux was recorded at 0.22 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> and 0.55 mg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup> in the CCP1 treatment of the wheat growing and barley growing season, respectively (Fig. 5.2C). The N<sub>2</sub>O flux in the barley growing season was 237% (P1), 184% (P2), 573% (CP1), 696% (CP2), and 725% (CCP1) higher than that in wheat growing season. Compared with P1, P2 had a 67% and 61% lower N<sub>2</sub>O flux in the wheat and barley growing seasons, respectively (Fig. 5.2C(1)).

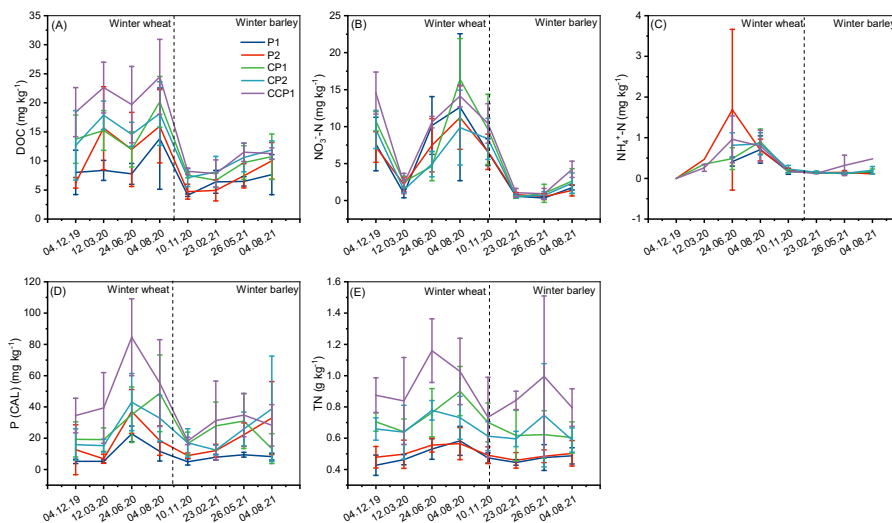
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In contrast, combined application of compost and P stimulated the  $\text{N}_2\text{O}$  flux at the higher P level, i.e., the CP2 treatment had an  $\text{N}_2\text{O}$  flux 41% and 30% higher in the wheat and barley growing season, respectively, than P2 (Fig. 5.2C(3)).

### 5.3.3 Soil nutrient retention

The factors sampling time, compost, and P fertilization significantly affected soil DOC, P-CAL, SOC, TN, and total phosphorus (TP) content (Table S5.3), of which DOC showed seasonal variations (Fig. 5.3). The soil content of DOC in the wheat season was always higher than in the barley season at the same sampling stage (Fig. 5.3A). In the wheat season, compost application significantly increased mean soil DOC content in the CCP1 treatment by 124% compared to P1. In contrast, the effect of compost on soil DOC content in the barley season was insignificant (Fig. 5.3A).

The soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  content changed over the season due to fertilization, plant nutrient uptake, and nitrification/denitrification. Compost addition or extra P fertilization had no additional effect on soil  $\text{NH}_4^+\text{-N}$  content, but it was associated with higher soil  $\text{NO}_3^-\text{-N}$  content in the wheat season (Fig. 5.3B, C). During the whole wheat season, the content of P-CAL did not show considerable seasonal variation in any of the treatments. However, the soil of the CCP1 treatment contained significantly more P-CAL than the treatments without (P1, and P2) or intermediate compost (CP1 and CP2) application. The contents of SOC, TN and TP remained relatively stable during the wheat and barley seasons (Fig. 5.3E, F and G). Compared with P1, double compost addition (CCP1) significantly increased the SOC and TN content by 103% and 87%, respectively. However, neither compost nor P addition caused a significant effect on TP content.



**Figure 5.16** Seasonal variation of soil DOC (A),  $\text{NO}_3^-\text{-N}$  (B),  $\text{NH}_4^+\text{-N}$  (C), and P-CAL (D), SOC (E), TN (F), TP (G) content in different period. Different lowercase letters on the same sampling date indicate a significant difference between treatments at  $P < 0.05$ . < LOQ indicates that the content of  $\text{NH}_4^+\text{-N}$  is less than  $0.03 \text{ mg kg}^{-1}$ . (1) and (2) indicate the wheat and barley season, respectively.

**Table 5.11** Results of repeated measures ANOVA for the main effects of compost (C), phosphorus (P), and their interactions on weight of leachate, TN in leachate and DOC, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, P-CAL (plant-available P) in leachate and soil.

		Leachate			Soil		
		P	C	P * C	P	C	P * C
DOC	<i>P</i>	0.624	0.187	0.633	0.213	<b>&lt;0.001</b>	0.550
	<i>F</i>	0.250	1.865	0.237	1.682	16.025	0.373
	<i>Eta</i>	0.015	0.189	0.015	0.095	0.667	0.023
NO <sub>3</sub> <sup>-</sup> -N	<i>P</i>	0.605	0.147	0.724	0.187	0.125	0.478
	<i>F</i>	0.278	2.169	0.129	1.896	2.376	0.527
	<i>Eta</i>	0.017	0.213	0.008	0.106	0.229	0.032
NH <sub>4</sub> <sup>+</sup> -N	<i>P</i>	0.605	0.147	0.724	0.139	0.619	0.343
	<i>F</i>	0.278	2.169	0.129	2.426	0.494	0.954
	<i>Eta</i>	0.017	0.213	0.008	0.132	0.058	0.056
P-CAL	<i>P</i>	0.263	0.525	0.402	0.251	<b>&lt;0.001</b>	0.141
	<i>F</i>	1.349	0.671	0.740	1.421	16.323	2.393
	<i>Eta</i>	0.078	0.077	0.044	0.082	0.671	0.130
TN	<i>P</i>	0.819	0.333	0.918	0.904	<b>&lt;0.001</b>	0.599
	<i>F</i>	0.054	1.179	0.011	0.015	24.606	0.288
	<i>Eta</i>	0.003	0.128	0.001	0.001	0.755	0.018
Weight	<i>P</i>	0.699	0.110	0.617			
	<i>F</i>	0.155	2.539	0.260			
	<i>Eta</i>	0.010	0.241	0.016			
SOC	<i>P</i>				0.721	<b>&lt;0.001</b>	0.296
	<i>F</i>				0.132	30.287	1.166
	<i>Eta</i>				0.008	0.791	0.068
TP	<i>P</i>				0.947	<b>&lt;0.001</b>	0.362
	<i>F</i>				0.005	13.261	0.880
	<i>Eta</i>				0.001	0.624	0.052

Eta: Partial Eta Squared

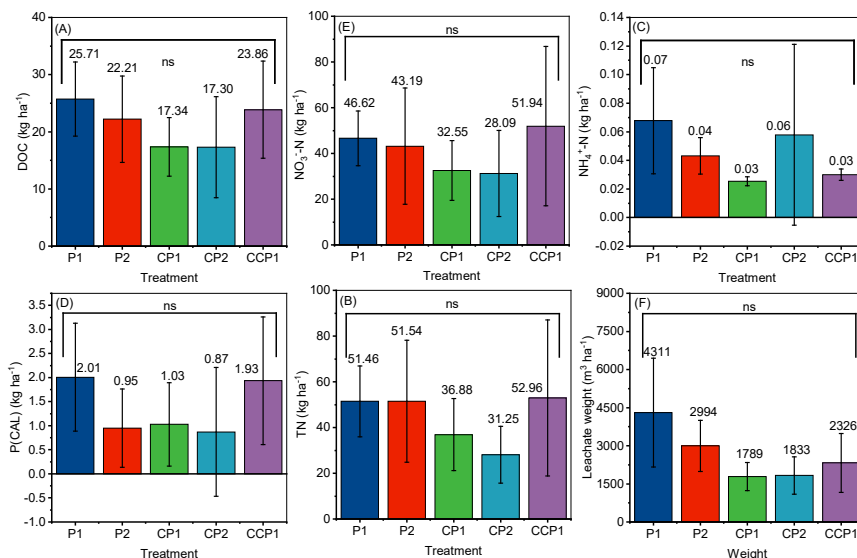
**Table 5.12** Results of Two-way ANOVA for the main effects of compost (C), phosphorus (P), and their interactions on total aboveground biomass, grain yield of wheat and barley.

Crop	Production		P	C	P * C
Winter wheat	Aboveground biomass	<i>P</i>	0.748	<b>&lt;0.01</b>	0.388
		F	0.107	10.74	0.786
		Eta	0.007	0.573	0.047
	Grain yield	<i>P</i>	0.412	<b>&lt;0.01</b>	0.262
		F	0.71	6.597	1.35
		Eta	0.042	0.452	0.078
Winter barley	Aboveground biomass	<i>P</i>	0.274	<b>&lt;0.01</b>	0.552
		F	1.281	6.719	0.369
		Eta	0.074	0.456	0.023
	Grain yield	<i>P</i>	0.316	<b>&lt;0.01</b>	0.427
		F	1.07	6.422	0.664
		Eta	0.063	0.445	0.04

Eta: Partial Eta Squared



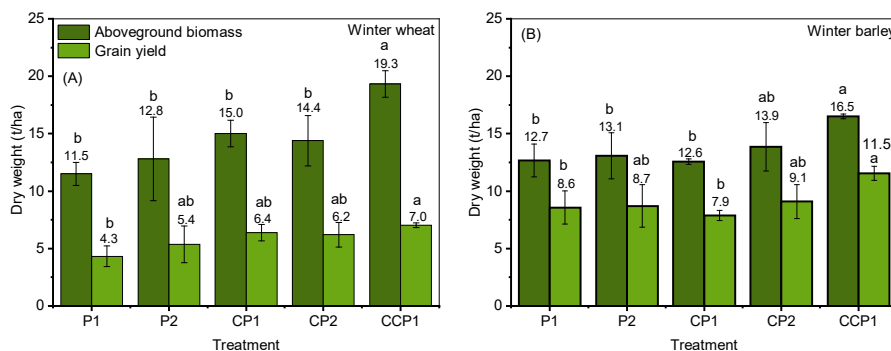
### 5.3.4 Nutrient leaching



**Figure 5.17** Mean content of DOC (A), NO<sub>3</sub><sup>-</sup>-N (B), NH<sub>4</sub><sup>+</sup>-N (C), P-CAL(D), TN (E) in leachate and leachate weight (F). Different lowercase letters in the same season indicate a significant difference between treatments at  $P < 0.05$ .

The DOC, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, P-CAL, and TN leaching amount between lysimeters amended with/without compost was insignificant (Fig. 5.4A, B, C, D, E). The total leachate weight over the whole experimental period of two years was in the order of P1 (4311 m<sup>3</sup> ha<sup>-1</sup>) > P2 (2994 m<sup>3</sup> ha<sup>-1</sup>) > CCP1 (2326 m<sup>3</sup> ha<sup>-1</sup>) > CP2 (1834 m<sup>3</sup> ha<sup>-1</sup>) > CP1 (1789 m<sup>3</sup> ha<sup>-1</sup>) (Fig. 5.4F). The results of the repeated-measures ANOVA showed that neither compost addition and P fertilization, nor their interaction, had a significant effect on DOC, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, P-CAL, and TN leaching (Table 5.3).

### 5.3.5 Crop production



**Figure 5.18** Production of aboveground biomass and grain yield in wheat season (A) and barley season (B). Different lowercase letters indicate significant differences between the treatments in the same growing season at  $P < 0.05$ .

Compost significantly increased aboveground biomass and grain yield in both the wheat and barley season (Table 5.5). But the interaction between compost and P application on plant growth was insignificant (Table 5.4). In the wheat and barley seasons, the aboveground biomass of lysimeters of the CCP1 treatment was significantly larger than the other treatments, reaching 19.3 and 16.5 t ha<sup>-1</sup>, respectively (Fig. 5.5B).

## 5.4 Discussion

### 5.4.1 Effect of temperature and precipitation on GHG emissions

Numerous studies have shown that compost addition significantly affected GHG emissions, however, the conclusions are still inconsistent, attributing the effects to differences in climatic conditions, soil physicochemical properties, and the application of artificial fertilizer (Ginting et al., 2003; Montemurro et al., 2006; Santos et al., 2021).

Temperature and precipitation are two critical factors affecting GHG emissions (Liu et al., 2017a; Schindlbacher et al., 2004). The majority of soil CO<sub>2</sub> emissions are associated with microbial biomass and metabolism, and the process is controlled by both temperature and moisture. Temperature can affect the release of nutrients by affecting SOM decomposition, and soil moisture is closely related to the availability of O<sub>2</sub> and substrate in the soil pore space. Based on continuous measurements of the CO<sub>2</sub> efflux, temperature and moisture of the soil at a ponderosa pine plantation in the Sierra Nevada Mountains in California (USA), Qi and Xu (2001) developed two analytical procedures to separate the effects of soil temperature and moisture and found that the relationship between soil CO<sub>2</sub> efflux and temperature could be well described using a power function. Hill et al. (2021) revealed that, compared with moisture,

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temperature was the primary driver of soil CO<sub>2</sub> emission in forest and grassland soil in Maryland (USA). However, this relationship is modified by soil moisture, which affects only the coefficient, but not the exponent, of the power function (Qi and Xu 2001). In our experiment, we found higher CO<sub>2</sub> emission rates in the barley growing season, although the mean air temperature in the wheat growing season was higher (14.17 °C) than in the barley growing season (12.98 °C), while the precipitation amount in the barley growing season (326.1 mm) was 134.87% higher than that in the wheat growing season. This suggests, in contrast to the findings above, that soil moisture rather than soil temperature exerted the primary influence on soil respiration in this temperature range and in this soil. Due to the drier conditions in the wheat growing season, many microbial processes reliant on water were limited, hence inhibiting microbial activity. With increasing soil water content, substrates concentrated in soil aggregates can diffuse to places of consumption, thereby increasing microbial activity and biomass, which in turn enhances microbial respiration (Qiu et al., 2005).

The contribution of soil inorganic carbon (SIC) to CO<sub>2</sub> emissions is usually neglected, because SIC is generally considered to be very stable (Raza et al., 2021). However, changes in soil properties due to intensive agricultural activities may lead to more abiotic CO<sub>2</sub> emissions. In our experiment, the soil pH value was about 7.5. Previous research reported that soil CaCO<sub>3</sub> can be a major source of abiotic CO<sub>2</sub> emissions in calcareous soils at pH 6.5-8.5 (Raza et al., 2021), suggesting that the contribution of CaCO<sub>3</sub> to soil CO<sub>2</sub> efflux is another potential reason to explain the much higher CO<sub>2</sub> efflux in the barley growing season. As described above, the higher CO<sub>2</sub> flux was accompanied by higher precipitation in the barley growing season compared to the wheat growing season. The higher precipitation, especially in summer 2021, might have leached any kind of acidic compounds, be it from nitrification (nitric acid), mineralization or root exudation (organic acids), to greater depth, and by this might have led to the increased decomposition of CaCO<sub>3</sub>, leading to increased soil CO<sub>2</sub> emission.

Well-drained, aerobic soils have been identified as significant atmospheric CH<sub>4</sub> sinks (Papen et al., 2001; Sadasivam and Reddy, 2014). Negative net CH<sub>4</sub> fluxes are expected when the activity of methanotrophs exceeds that of methanogens in soil (Conrad, 2009; Dutaur and Verchot, 2007). The most important factor affecting the CH<sub>4</sub> flux is soil moisture content, as it determines the O<sub>2</sub> diffusion into soil (Yavitt et al., 1995). Soil moisture may also reduce the diffusion of CH<sub>4</sub> to methanotrophic microorganisms (Van den Pol-van Dasselaar et al., 1998). In contrast, CH<sub>4</sub> production is positively correlated with soil moisture. High soil moisture lowers the availability of O<sub>2</sub>, creating strictly anaerobic sites (Yavitt et al., 1995), with increased formation of CH<sub>4</sub> by methanogens. In our experiment, a lower net CH<sub>4</sub> uptake rate (less negative CH<sub>4</sub> flux) was observed in the barley growing season compared to the wheat growing season. This is very likely due to the higher precipitation in the barley growing season, which either might have made the conditions less favorable for CH<sub>4</sub> oxidation, or might have improved the

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conditions for CH<sub>4</sub> production, or both at the same time. However, under aerobic conditions, CH<sub>4</sub> produced in anaerobic parts of the soil can be oxidized by methanotrophs (Segers, 1998). Furthermore, the temperature is essential, as both CH<sub>4</sub> oxidation and production are microbial processes (Czepiel et al., 1995; Dobbie and Smith, 1996; Van den Pol-van Dasselaar et al., 1997), and the optimum temperature for CH<sub>4</sub> production is higher than that for CH<sub>4</sub> oxidation in the soil (Nesbit and Breitenbeck, 1992).

Nitrous oxide is a trace GHG contributing to global warming and stratospheric O<sub>3</sub> depletion. Production of N<sub>2</sub>O is mainly controlled by soil aeration and the availability of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. Higher N<sub>2</sub>O emission occurred in the barley growing season which was characterized by higher precipitation than the wheat growing season. Previous research reported that N<sub>2</sub>O emitted from fields amended with N fertilizer is mainly affected by soil moisture conditions (Liu et al., 2017a), which is consistent with our research. In well-aerated soils, nitrification is the dominant process as O<sub>2</sub> availability is non-limiting. However, compared with well-aerated soils, the activity of soil denitrifiers is increased in wet soils, leading to higher N<sub>2</sub>O emission from denitrification (Qin et al., 2020). Under water-saturated soil conditions, though, the denitrification-related N<sub>2</sub>O:N<sub>2</sub> molar ratio will be decreased due to stimulation of complete reduction of N<sub>2</sub>O to N<sub>2</sub> (Muhammad et al., 2020).

#### **5.4.2 Effect of compost on GHG emissions**

The young recultivation soil in this area is unique and characterized by a low C and nutrient (such as N and P) content and microbial biomass. Compost is a humic-like material that acts as an organic additive increasing SOC, TN, and TP, and is expected to improve mineral N fertilizer utilization efficiency by optimizing soil aggregation, BD, moisture content, and stimulating the development of microbial biomass (Huang et al., 2010). Hence, the application of compost has a tremendous potential to improve recultivation soil quality. Compost decomposition will result in the release of available C as energy source of soil microorganisms. Previous studies have shown that the application of compost favors the activity of microbial communities, leading to the depletion of O<sub>2</sub> and favoring the production of CO<sub>2</sub> (Fabrizio et al., 2009; Ginting et al., 2003; Grigatti et al., 2020; Sorrenti and Toselli, 2016). In contrast, consistent with our first hypothesis, compost addition caused no significant increase in CO<sub>2</sub> emission, neither in the wheat nor in the barley growing season, when co-applied with P. Labile C is an important source for the microbial activity and metabolism. Although part of the organic C is taken up directly by microorganisms and emitted through respiration, a larger portion will become part of the microbial biomass and contributes to the effective (re)circulation of C and other elements in the soil-plant system under suitable N:P conditions. Moreover, due to the specificity of this young arable soil, the microbial biomass of the soil is comparably lower than in nutrient-rich soils, which means that the efficiency of SOM utilization is low (Li et al., 2021a). On this basis, we

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estimate that the SOM in the soil will be retained in a stable form at the start of reclamation. In addition, the small molecule DOC absorbed by plant root may be another way to regulate soil CO<sub>2</sub> emissions. Furthermore, sole P application caused no significant effect on the CO<sub>2</sub> emission, though P is an essential factor affecting microbial activity. This is due to the lack of primary nutrients such as SOM, mineral N, and available P for microbial activity in young recultivation soils, which limits the impact of P addition on improving microbial community and activity (Li et al., 2021a).

Soil CH<sub>4</sub> flux is controlled by biotic and abiotic factors simultaneously (Smith et al., 2018), and the CH<sub>4</sub> flux is the result of the balance of methanogenesis and methanotrophy (Conrad, 2009; Dutaur and Verchot, 2007). Compost and P application affected the CH<sub>4</sub> flux in this experiment, but the effect was insignificant. The nutrient released from the compost to the soil provides an energy resource for the soil microorganisms, which contributes to the enhancement of their activity, increases the consumption of O<sub>2</sub> and the formation of anaerobic conditions (Ros et al., 2006; Wild et al., 2010). However, at the same time the application of compost increases the porosity of the soil to a certain extent (Celik et al., 2010; Rivenesshield and Bassuk, 2007), which in turn enhances the aeration of the soil, and thereby increases the activity of methanotrophs under aerobic conditions. Thus, the effect of compost and P fertilization on biotic and abiotic processes apparently balanced each other.

To date, several studies have analyzed the effect of high C soil additives on soil N<sub>2</sub>O emission, but there is still no uniform conclusion (Favoio and Hogg, 2008; Li et al., 2021b; Wei et al., 2020). Consistent with our first hypothesis, compost application had no significant effect on soil N<sub>2</sub>O emission. On the one hand, the enhanced C input provides the energy source required for the activity of denitrifying bacteria, which is conducive to the generation of N<sub>2</sub>O. However, at the same time, to retain a constant C:N ratio, microorganisms are forced to take up available N from the soil to build up microbial biomass (Cleveland and Liptzin, 2007). Thus, this process will facilitate the conversion of N from mineral to organic state, which reduces the N source required for the denitrification process, thereby mitigating the risk of increasing N<sub>2</sub>O emission. Soil P availability has been shown to affect N<sub>2</sub>O emission by affecting the growth and activity of microbial biomass. However, so far there is no consensus. Some studies report that P addition can decrease N<sub>2</sub>O emission by stimulating microbial P retention (Hall and Matson, 1999; Sundareshwar et al., 2003), while others found greater N losses via N<sub>2</sub>O in response to P addition in field experiments (Mehnaz and Dijkstra, 2016; Mori et al., 2010). In our experiment, P application or co-application of P with compost had no significant effect on N<sub>2</sub>O emission, implying that soil microorganisms (nitrifiers or denitrifiers) were not P-limited in this soil.

#### **5.4.3 Effect of compost on soil nutrient retention**

Compost is a humic-like material that can be used as an HCA (Fabrizio et al., 2009). It serves

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as an abundant C source for soil microorganisms, which is beneficial to soil nutrient retention (Fan et al., 2019). Therefore, compost application is regarded as an effective method to improve soil fertility and crop yield, though it is rarely used in intensive agriculture in Europe (Viaene et al., 2016). In our experiment, application of compost led to an increase in soil P availability. This is because SOM can have a synergistic effect on P availability for various reasons: first, the release of inorganic P due to the mineralization of SOM in compost which is rich in P; second, release of organic anions that may compete for P adsorption sites, thereby improving P availability in soil and fertilizers (Hue, 1991); third, P adsorption can be reduced due to the increase of soil pH caused by the decomposition (hydrolysis) of SOM (Haynes and Mokolobate, 2001). In line with the second hypothesis, compost application led to a higher SOC, TN, and TP content. Hence, our experimental results confirmed the previous conclusions that compost is a suitable additive to improve soil nutrient content as well as soil crop quality and quantity (Guo et al., 2019; Mylavarapu and Zinati, 2009). Therefore, the positive effect of compost application on soil C, N, and P availability and utilization emphasizes its potential for making agriculture sustainable in the long term (Viaene et al., 2016).

#### **5.4.4 Effect of compost on soil nutrient leaching**

Previous research showed that the increase in SOM along with the decomposition of compost might bear the risk of nutrient leaching (Sorrenti and Toselli, 2016). In addition, compost application can reduce the BD of the soil and increase soil porosity, which may offset the positive effect on N retention (Wong et al., 1999). In contrast, Brown and Cotton (2011) reported compost can increase the WHC and decrease nutrient leaching. Consistent with our third hypothesis, we found no significant increase in N and P leaching after the addition of compost and mineral N and P fertilizers. Even at the higher P fertilization level, we found no increase in  $\text{NO}_3^-$  leaching, although P application increased soil DOC and  $\text{NO}_3^-$ -N content (Fig. 5.3A). A likely explanation is that the N released by SOM decomposition will be quickly taken up by microorganisms due to the C:N limitation of the microbial community (Cleveland and Liptzin, 2007), but also by the crops due to stimulated plant growth. That means that compost as a nutrient-rich additive can provide the required elements for microbial biomass growth, and thereby enhance nutrient retention in the soil (Lee et al., 2004).

#### **5.4.5 Effect of compost on crop production**

In line with the fourth hypothesis, our findings indicated that the double amount of area-typical compost application ( $80 \text{ t ha}^{-1}$ , CCP1) significantly increased total aboveground biomass and grain yield of wheat and barley relative to the P1 treatment. This promotion of crop performance is consistent with previous studies, and can be very likely attributed to improved soil nutrient availability and quality (Abbasi et al., 2002; Ibrahim et al., 2008), but also enhanced soil water

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availability. Previous studies have shown that compost addition to soil can improve WHC and soil aggregate stability (Tejada and Gonzalez, 2003), and can also accelerate germination and increase crop growth during the growing season (Agegnehu et al., 2016). In addition, DOC derived from the mineralization of compost could further stimulate microbial growth and activity, and lead to an enhanced microbial mining of nutrients from both compost and SOM, as microorganisms will be forced to assimilate soil nutrients (N, P, S) along with the DOC they take up to build up their biomass (Cleveland and Liptzin, 2007; Reichel et al., 2018). This could in turn also lead to a higher nutrient availability for plants.

## **5.5 Conclusions**

Combined application of compost and mineral N and P fertilizer in our two-year outdoor lysimeter experiment resulted in an increase in aboveground biomass and grain yield of both winter wheat and winter barley, but did not significantly increase GHG emissions and nutrient leaching. In addition, soil contents of key variables, such as SOC,  $\text{NO}_3^-$ , and P-CAL, increased, while the increased P availability did not lead to faster compost decomposition and nutrient losses from this particular soil in our study. We conclude that combined compost and mineral fertilizer application, especially with higher amounts of compost, can mitigate the negative effects of climate change by storing more C than it releases through GHG emissions, increasing soil WHC and productivity, and preventing the leaching of nutrients into groundwater and surface water, even after heavy rainfall. However, more long-term studies with different soils and compost application rates are needed to make generalized statements.

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## Chapter 6

### Synopsis



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## 6.1 Summary

To date, although many studies have been conducted to research the effect of HCA on soil N retention, few studies have comprehensively investigated the interaction between HCA, soil type, and available P levels on  $\text{N}_2\text{O}$  emissions,  $\text{NO}_3^-$  leaching, and crop yield. In this thesis, two incubation experiments, one lysimeter experiment, and one meta-analysis were conducted to evaluate the role of HCA in improving soil N retention in different soils with different P content, which is related to climate change, environmental pollution, and agricultural production.

The first part of the PhD thesis presents the current state of knowledge on  $\text{N}_2\text{O}$  production pathways and influencing factors, the direct and indirect effects of HCA on soil N retention, crop yield, and aboveground biomass. In addition, we have also introduced 1) why making appropriate use of the N source after harvest and balancing the N input and uptake is essential for mitigating the N losses and increasing crop yield and 2) the advantages of lysimeter experiments compared to a specific field or incubation experiments.

Nitrous oxide emission and  $\text{NO}_3^-$  leaching are two critical pathways of soil N losses. In Chapter 2, we identified 345 observations from 90 peer-reviewed articles on a global scale and comprehensively assessed the effects of crop residues on  $\text{N}_2\text{O}$  emissions and  $\text{NO}_3^-$  leaching from agricultural fields through a meta-analysis. The analysis included ten factors: climatic conditions, land use type, soil pH, soil texture, synthetic fertilizer component and type, as well as the application times, crop residue type, tillage, and the duration of the experiments. Linear or logarithmic regression analysis showed that MAP and MAT significantly influenced the effect of crop residues on  $\text{N}_2\text{O}$  emission from agricultural fields. At the same time, soil C, N, and P content were key variables influencing  $\text{NO}_3^-$  leaching from crop residues. The meta-analysis results revealed that adding crop residues significantly increased  $\text{N}_2\text{O}$  emissions in temperate regions and upland soils but decreased them in paddy soils. In addition, crop residues significantly mitigated  $\text{NO}_3^-$  leaching in both paddy and upland soils. Soil pH and texture were also important factors affecting the influence of crop residues on soil N retention. Crop residues remaining on or returned to the field significantly increased  $\text{N}_2\text{O}$  emissions in both acidic (pH 5.5-6.5) and alkaline (pH > 7.5) soils but mitigated  $\text{NO}_3^-$  leaching in neutral (pH 6.5-7.5) soils. Return of crop residues caused a particularly strong and significant increase in soil  $\text{N}_2\text{O}$  emissions except for soil with clay texture, indicating that clay content was a critical determinant of the soil  $\text{N}_2\text{O}$  emission response to crop residue application. In contrast,  $\text{NO}_3^-$  leaching from silty clay loam, sandy loam, and silt loam showed a negative response to crop residue application. We found that neither the composition nor the form of nitrogen fertilizer affected the effect of straw on  $\text{N}_2\text{O}$  emissions. Compared with NPK fertilizer, crop residue application significantly decreased  $\text{NO}_3^-$  leaching from soil treated with N fertilizer only (especially  $\text{NH}_4\text{NO}_3$ ). In addition, when the crop residues were only applied once, the effect of

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mitigating leachate leaching was significant. The application of low C/N residues, maize straw and wheat straw stimulated N<sub>2</sub>O emission. In addition, wheat straw had a significant effect on mitigating NO<sub>3</sub><sup>-</sup> leaching. Higher N<sub>2</sub>O emission occurred in no or reduced tillage areas and in experiments of short duration. The results revealed that appropriate crop residue management adapted to the site-specific soil and environmental conditions is critical for increasing SOC stocks and decreasing nitrogen losses.

Chapter 3 presented the effects of HCA (wheat straw, sawdust, and leonardite) on soil N retention in soils with different textures and P levels in an incubation experiment. All soils tested (nutrient-poor silty soil, nutrient-rich silty soil, and nutrient-rich sandy soil) received the same amount of N fertilizer, and then each soil received different HCA types and P fertilizer levels. The results of a stepwise multiple regression analysis showed that incubation time, soil types, and HCA were essential factors that significantly controlled the efflux of CO<sub>2</sub> and N<sub>2</sub>O. In contrast to sawdust and leonardite, the co-addition of wheat straw with P fertilizer positively affected soil microbial activity (indicated by CO<sub>2</sub> emissions) in soils with high nutrient content. In nutrient-poor soil, none of the treatments significantly affected N<sub>2</sub>O emission, suggesting that soil condition is an essential factor in regulating the dynamics and stability of SOM in soils. In addition, HCA had a positive effect on NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> immobilization and MBC content in soils with high nutrient content. Simultaneously, this effect was influenced by the decomposition rate of HCA, which is associated with the input of labile C into the soil.

Further experiments described in Chapter 4 extended the study in Chapter 3 by investigating the effect of <sup>13</sup>C-labeled glucose and HCA on soil N retention. All samples were placed in an incubator to simulate the local average ambient air temperature dynamics from September to April. The incubation temperatures were adjusted based on the monthly average air temperature of the past five years in the sampling area: 15.2°C (September), 12.9°C (October), 7.1°C (November), 5.9°C (December), 3.4°C (January), 4.1°C (February), 6.8°C (March), and 10.2°C (April). Repeated measures ANOVA was used to test the effect of HCA, glucose, and P on soil GHG emissions, soil nutrient content, microbial biomass, and the <sup>13</sup>C recovery in MBC. The results showed that HCA application increased the average content of DOC in the soil and promoted the growth of microbial biomass, which was beneficial for mitigating NO<sub>3</sub><sup>-</sup> leaching. In addition, glucose had a positive effect on reducing NO<sub>3</sub><sup>-</sup> content in HCA-amended soils. HCA reduced the <sup>13</sup>C content of microbial biomass, while adding P had the opposite effect. The combined addition of a readily available C source and more recalcitrant HCA may improve the efficiency of N retention in post-harvest soils, especially for more recalcitrant HCA types like sawdust, i.e., the combination of glucose with sawdust may delay the release of microbially retained N.

The two-year lysimeter experiment described in Chapter 5 studied the response of soil GHG emissions, N retention, and crop yield to different compost and P application levels. The results

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showed that compost application significantly increased the aboveground biomass and grain yield of winter wheat and winter barley, particularly in the double compost treatment. Combined application of compost, mineral N, and mineral P had no significant effect on GHG emissions in the winter wheat and winter barley growing seasons. Adding the double amount of compost increased TC, DOC, and soil nutrient contents, especially TN, TP, and P-CAL, compared with the control, but did not lead to a significant increase in GHG emissions and DOC,  $\text{NO}_3^-$  and P leaching. In contrast, bare soil had the highest leaching losses of DOC and  $\text{NO}_3^-$  compared to all plant-covered treatments. The experiment revealed that combined compost and mineral N fertilizer application was a suitable management option for increasing crop yield and reducing its variability without significantly increasing GHG emissions and leaching losses of  $\text{NO}_3^-$  and P in nutrient-poor soil.

## 6.2 Synthesis

HCA application has great potential to improve soil N retention and crop yield. Soils differ in particle size distributions (texture), aggregate stability, nutrient content, and aerobic conditions, which has implications for the mineralization of SOM and hence for the nutrient supply to soil microbes (Hassink, 1992). Restricted by the C:N:P stoichiometry, the physicochemical properties of HCA are critical for controlling the effect of the extra C input on microbial activity and nutrient release and/or retention (Huang, 2004). Based on previous research (Steiner et al., 2008; Reichel et al., 2018; Zhou et al., 2020), it was evident that conducting comprehensive research accessing the effect of HCA on soil N retention for different soil conditions is necessary. In this thesis, a meta-analysis and three experiments were designed and conducted, providing strong evidence that soil texture, physicochemical properties of HCA, and the soil P content, e.g., modulated by different P fertilizer levels, are important factors governing soil N retention and crop yield.

Studies on the effect of HCA return on soil N retention have been frequently conducted in recent years (e.g., Hepperly et al., 2009; Homyak et al., 2017), as well as related meta-analyses (e.g., Chen et al., 2013). However, a comprehensive assessment of the effect of HCA return on soil N retention under all environmental conditions, such as climatic zones, soil types and textures, and agricultural management strategies, has not been available until now. In Chapter 2, we integrated studies on the impact of crop residue application on  $\text{N}_2\text{O}$  emissions and  $\text{NO}_3^-$  leaching through a meta-analysis, and our statistical results showed that the effect of crop residues on soil N retention is affected by a combination of climatic conditions, soil properties, crop residue type and agricultural management (fertilization and tillage). In terms of climatic conditions, MAT and MAP control microbial nitrification and denitrification and subsequent N losses significantly by modifying soil moisture and  $\text{O}_2$  content, as well as the enzyme activities of soil microbes (Barnard et al., 2006; Exenberger and Pondorfer, 2011; Prentice et al., 2012).

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Soil physical and chemical properties, differing, e.g., in BD, WHC, soil texture, nutrient content, and pH, are other essential factors controlling soil N retention (Bollmann and Conrad, 1998). In general, the labile C input was constrained by the C:N ratio and decomposability of HCA. For example, compared with sawdust with a C:N ratio between 100 to 400, wheat straw (C:N ratio 50-100) contains less recalcitrant compounds, leading to higher degradation rates and higher C input, which is beneficial for mitigating N losses (Cleveland and Liptzin, 2007; Kostov et al., 1991; Reichel et al., 2018).

Cropland management strategies control the availability of soil N, P, and K (Malghani et al., 2010). In addition, soil nutrient contents and their distribution in the soil significantly correlate with the development of the microbial community (Chen et al., 2013). The statistical results of our meta-analysis revealed that crop straw returned to the field increased N<sub>2</sub>O emissions on average by 29.7% and reduced NO<sub>3</sub><sup>-</sup> leaching on average by 14.4%, further validating the conclusion that crop straw is beneficial for improving soil quality. Therefore, the results of our analysis are instructive on how to use HCA in different environments.

As presented in Chapter 3, the first experiment was conducted in a full-factorial, completely randomized design of three soils × one N level × three P levels × four C amendments, resulting in a total of 36 treatments. The nutrient availability in the soil controls microbial community development and enzyme activities (Schmidt et al., 1999). Hence, two opposite hypotheses could be proposed: 1) the recultivation soil (PUS) was characterized by a low abundance of soil microorganisms, as well as low labile C and available P content, and therefore might not exhibit a significant response to HCA addition; 2) due to the low nutrient and SOC content of PUS, the microbial biomass might respond to HCA addition particularly strongly. Consequently, our first hypothesis was confirmed that HCA and P addition caused no significant effect on soil GHG emissions in PUS soil, which supported the previous consensus that the N dynamics of soil amended by HCA are affected by the original biogeochemical properties of the soil (Li et al., 2021a). Similar to our research, Wisethaksorn et al. (2020) found that the effect of HCA application on soil N dynamics in an organically managed nutrient-poor paddy soil system was insignificant, as well as in the organic rice cultivation system.

The properties of HCA, especially the C:N ratio and decomposability, are critical in determining the effect of HCA on N retention (Reichel et al., 2018; Li et al., 2021a). The experiment in this thesis showed that both nutrient-rich soils (RUS and RSS) amended with wheat straw (C:N) had significantly higher CO<sub>2</sub> emission (an indicator of microbial activities) and MBC content compared with the corresponding sawdust treatment. As a by-product of lignite mining, leonardite is the coal with the lowest degree of carbonization. Compared with wheat straw, leonardite has a similar C:N ratio, but high humic acid content and lower moisture content (Ricca et al., 1993). In addition, leonardite has a highly aromatic structure, which makes it biochemically more stable, so that it is difficult to undergo chemical changes and be utilized by

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soil microorganisms after being applied to the soil (Darley and Gray, 1988). Hence, due to its limited decomposability, the addition of leonardite caused no significant short-term effect on microbial activity and N retention.

Previous research showed that soil texture (depending on the percentage of sand, silt, and clay) is an essential factor affecting microbial activity and the development of the microbial community (Hamarashid et al., 2010). For example, clay soil with higher WHC is associated with higher nutrient retention and less N leachate potential (Dempster et al., 2012). As a result, these findings support our result that wheat straw increased  $\text{N}_2\text{O}$  emission in RUS (silty texture) more than in RSS (sandy texture).

Besides soil texture, the co-application of P with HCA affecting N retention was assessed in this experiment. The results showed that combined addition of P and wheat straw increased  $\text{CO}_2$  emissions from the RUS soil but simultaneously decreased the mineral  $\text{NO}_3^-$  content, indicating that the availability of P fertilizer stimulated the effect of HCA application on N retention in this soil. In addition, due to the C input through the decomposition of the substrate, the HCA application caused a significant increase in DOC content in RSS. Hence, our research results indicate that a combination of soil properties and C availability influences the impact of P addition on GHG emissions and nutrient retention.

Experiment 1 in Chapter 3 showed that adding HCA did not change GHG emissions and nutrient content of the recultivated soil. Experiment 2 in Chapter 4 was designed to optimize soil N retention over a simulated winter period and re-release under spring temperature conditions by adding and stimulating the decomposition of HCA (wheat straw and sawdust) under realistic soil temperature and moisture conditions. In accordance with previous publications (Curtin et al., 1998; Wang et al., 2019a; Wu et al., 2013), our experiment showed a higher  $\text{CO}_2$  emission, which is an indicator of microbial activity, after the addition of HCA. The most likely explanation is that labile C provides the necessary resources for the development of the soil microbial community and stimulates microbial activity (Wei et al., 2019). The development of soil microbial biomass, reflected by an increase in MBC and MBN, can increase N retention in the soil, thereby mitigating the risk of N leaching (Yao et al., 2011). As an additional C source in the soil, microorganisms can rapidly use glucose, which further increases the intensity of N conversion from inorganic (mineral) to organic form (Xu et al., 2020).

There is a consensus that the function of P on soil N retention is controlled by the match or mismatch of soil and microbial C:N:P stoichiometry (Cleveland and Liptzin, 2007). As confirmed by our incubation experiment, which included HCA, glucose, and P treatments, in the HCA treatments with G or with P, the  $\text{NO}_3^-$  content was lower than in the corresponding HCA treatment (WS or SD) without G and P, and the combined HCA+G+P treatment had the lowest  $\text{NO}_3^-$  content at the end, albeit not significant in most cases. In addition, our results

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showed that glucose application did not contribute to soil GHG emissions, indicating that a substantial part of it was immobilized by microbial biomass, as also reflected in the increasing  $\delta^{13}\text{C}$  value of MBC with increasing incubation time. Thus, this result perhaps indicated that labile C is more readily available to microorganisms and forms stable substances that remain in the microbial community for a long time rather than being lost rapidly.

As a system for measuring the C and N balance and quantifying C and N losses with leachate and in gaseous form, lysimeters provide an intermediate link between laboratory and field conditions, combining their strengths and avoiding deficiencies. Compared to other simple lysimeters (Kay et al., 2005; Renaud et al., 2004), our lysimeters had a larger volume (1.1 m depth, 1 m<sup>2</sup>, or 0.5 m<sup>2</sup> surface area) and were placed in the surrounding soil, which means that the soil was less affected by external factors during the collection process and had a more stable microbial community and physical structure. In Chapter 5, a lysimeter experiment was carried out to investigate the effect of compost and P fertilizer application on soil N retention and crop yield. Our experimental results showed that compared with mineral P fertilizer application, compost or compost + mineral P application caused no significant increase in GHG emission. In contrast, it significantly increased the SOC, DOC, P-CAL, and TN content of the soil but not of the leachate. Hence, this experiment revealed that compost is an environmentally friendly HCA that can increase soil nutrient levels without increasing the risk of nutrient and DOC leaching.

Phosphorus accounts for about 0.2 percent of a plant's dry weight, where it is primarily a component of tissue molecules such as nucleic acids, phospholipids, and adenosine ATP. In the lysimeter experiment, we found that increasing the P fertilizer amount alone caused no significant positive effect on crop yield. However, combining the P fertilizer with compost significantly increased winter wheat and winter barley yields. The lysimeter experiment also showed that the amount of leachate from the bare soil treatment was significantly higher than from all other treatments. Therefore, the risk of nutrient loss from the soil is much higher in areas with bare soil, especially in winter. Hence, increasing soil vegetation cover is critical in mitigating soil nutrient loss. From all the research results obtained in this work, we concluded that combining the application of compost with mineral N and P fertilization is an agriculture management practice that allows for an increase of both crop yield and yield stability in an environmentally friendly way.

### **6.3 Perspectives**

Our experiments show the effect of HCA addition on soil N retention, as well as on the correlation between HCA type, decomposability, and soil N retention. Therefore, the results of this thesis can help to better understand the relationship between HCA properties and soil quality, environmental pollution, and crop yield. However, due to the time constraints of the

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experiments, some questions still need to be addressed. These questions are touched upon in the following.

### **6.3.1 Soil properties and ecosystems**

The initial conditions of the soil critically affect the decomposition of HCA. In this thesis, we investigated the effect of HCA addition on N retention in three different soil types, showing that soil chemical and physical properties are key factors affecting soil N retention after HCA addition. Although the effect of HCA on soil N dynamics has been demonstrated in this thesis, additional studies are necessary because soil types have profound variations at both the regional and the global scale. In addition, soils with higher nutrient content and microbial activity are generally more conducive to decomposing recalcitrant HCA. As a result, future research should consider soils rich in nutrients, with different textures, and C:N:P stoichiometry, pH, SOC, etc. The type of land use also influences the effects of HCA on soil N. For example, wheat straw application increased microbial N retention in upland soil (Yang et al., 2015). In contrast, wheat straw return to paddy soil caused higher  $\text{NO}_3^-$  leaching (Yang et al., 2015). Further studies should explore the effect of HCA application on N retention in different ecosystems, such as upland cropland, paddy soils, pasture, and forest. In this way, we can reach a broad conclusion on the relationship between HCA and soil N availability and its role in different terrestrial ecosystems.

### **6.3.2 Using $^{15}\text{N}$ to understand N fate in agroecosystems**

Stable isotope labeling technology is a powerful method to study the cycling of elements in the pedosphere-biosphere-atmosphere system. Our study investigated the effect of the interaction of  $^{13}\text{C}$ -labeled glucose and HCA on N retention at different P levels and observed the fate of  $^{13}\text{C}$  in the belowground compartment. Several studies have investigated the effect of C addition on soil microbial dynamics and soil N losses; however, most of these studies only focused on glucose but ignored its interaction with HCA (Chenu et al., 2001; Dungait et al., 2011). After eight months of incubation, we concluded that the efficiency of C utilization by soil microorganisms mainly depends on the decomposability of HCA substrates and available P resources.

It is consensus that the fate of soil N resources is closely coupled with the SOC dynamics (Anaya et al., 2007). Tracing the fate of applied N fertilizers in the soil-plant system has great potential to effectively improve the N use efficiency and decrease environmental pollution in sustainable agricultural development (Xia et al., 2017).  $^{15}\text{N}$ -labeled substances are widely used to study soil N dynamics (Barraclough, 1995). For example, Akkal-Corfini et al. (2010) investigated the effect of HCA addition on the dynamics of N mineralization, plant N uptake, and  $\text{NO}_3^-$  leaching, allowing the calculation of the efficiency of N fertilizer use. In conclusion,

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stable isotope labeling technology is needed to comprehensively assess N dynamics in soils, including leaching, sequestration, microbial utilization, and crop uptake. In the future, further studies combining the application of  $^{13}\text{C}$  and  $^{15}\text{N}$  will be necessary for a deeper understanding of the relationship between soil C and N resources at a micro-scale.

### **6.3.3 A meta-analysis focusing on the effect of biochar on N retention**

Several studies have been conducted to assess the effect of HCA addition on soil N retention (Reichel et al., 2018; Li et al., 2021a; Yang et al., 2015).  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching are significant N-loss pathways in agroecosystems (Li et al., 2021b). In this thesis, we evaluated the impact of crop residues on N retention globally. Compared with crop residues, biochar has higher stability and other physiochemical properties and, therefore, can have longer-term effects on N retention (Wu et al., 2013). Hence, analyzing the impact of biochar application on soil N dynamics and comparing the effect with crop residues has great potential to optimize the application of HCA for maximizing soil N retention and minimizing N losses.

Though a lot of meta-analyses have been conducted to summarize the effect of biochar on soil N dynamics, most of them focused on the individual impact (Schmidt et al., 2021). Hence, a detailed assessment of the combined effects of biochar with fertilizer or crop type is essential to understand the potential and far-reaching uses of biochar. Compared to previously published meta-analysis papers, influencing factors, e.g., soil sand content, soil porosity, C:N, crop varieties, etc., should be considered in more detail. To improve the accuracy of such a meta-analysis, the contribution of NO and  $\text{NH}_3$  emissions and  $\text{NH}_4^+$  leaching must be considered in addition to  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching. Finally, since soil N availability is closely related to crop yield, further studies will be needed to evaluate the effect of HCA on crop yield.



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## Appendix

**Table S2.1** Between-group variability (Qb) among observations (n) indicating their potential as predictive variables of N<sub>2</sub>O emission and NO<sub>3</sub><sup>-</sup> leaching response to crop residue application. A larger Qb value indicates a better predictor than a variable with smaller Qb. \*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001.

Variables	N <sub>2</sub> O emission		NO <sub>3</sub> <sup>-</sup> leaching	
	n	Qb	n	Qb
All	255		90	
Climate zone	244	9.1739*	76	0.0503
pH	254	8.3873*	82	11.6977**
Texture	234	39.2044***	62	4.0506
Fertilizer component	172	3.3883	75	3.4263*
Nitrogen fertilizer type	151	1.7844	71	0.2182
Application time	148	7.9708*	50	3.5734
Residues type	253	119.7044***	89	4.8838
Plowing depth	167	11.3567**	77	1.8921
Experimental duration	145	27.1575***	79	1.2252

**Table S2.2** Weighted response ratio ( $\ln RR_{++}$ ) of soil N<sub>2</sub>O emission for correlative variables with 95% bootstrap confidence interval (CI).

Variable	Categories	Number	$\ln RR_{++}$	Lower CI	Upper CI
Climate Zone	Total	255	0.30	0.12	0.13
	Tropical	67	0.03	-0.16	0.26
	Temperate	178	0.36	0.22	0.52
Landuse type	Upland soil	204	0.46	0.30	0.64
	Paddy soil	51	-0.18	-0.31	-0.04
pH	< 5.5	38	0.19	-0.15	0.69
	5.5 -6.5	80	0.54	0.27	0.85
	6.5 -7.5	57	0.11	-0.06	0.29
	> 7.5	80	0.28	0.13	0.47
Soil texture	Sandy clay loam	13	0.20	-0.11	0.61
	Silty clay loam	12	0.27	-0.39	1.74
	Loamy sand	6	0.48	0.28	0.79
	Loamy clay	4	0.15	0.06	0.25
	Sandy loam	20	0.64	0.26	1.12
	Sandy clay	8	0.50	0.26	0.85
	Silt loam	36	0.35	0.09	0.65
	Silt clay	15	0.86	0.09	1.68
	Clay loam	36	0.20	-0.08	0.59
	Loam	47	0.18	0.01	0.40
	Sand	24	0.13	-0.24	0.72
	Silt	4	0.59	0.02	1.43
	Clay	10	-0.32	-0.55	-0.01
Single or multi-nutrient fertilizer	N	65	0.19	-0.03	0.44
	NPK	108	0.01	-0.09	0.13
Nitrogen fertilizer type	Urea	124	0.07	-0.05	0.19
	NH <sub>4</sub> NO <sub>3</sub>	12	0.19	-0.36	1.08
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10	-0.02	-0.34	0.35
	NH <sub>4</sub> HCO <sub>3</sub>	6	-0.16	-0.31	0.03
Fertilizer application times	1	43	0.00	-0.19	0.25
	2	48	0.11	-0.05	0.28
	3	58	-0.04	-0.17	0.10
	4	10	-0.32	-0.53	-0.02
Residues	Low C/N residues	52	1.63	1.11	2.37
	Sawdust	2	-0.52	-0.73	-0.15
	Sorghum straw	2	0.27	-0.09	0.76
	Sugarcane straw	3	-0.75	-0.76	-0.73

	Barley straw	4	0.05	-0.11	0.24
	Cotton straw	2	0.70	-0.26	2.85
	Maize straw	28	0.35	0.14	0.61
	Rice straw	51	-0.17	-0.30	-0.02
	Rye straw	4	-0.18	-0.53	0.65
	Wheat straw	102	0.19	0.06	0.36
Plow (cm)	0	42	0.35	0.11	0.67
	0 – 10	56	0.74	0.39	1.22
	10 – 20	66	0.14	-0.02	0.36
	> 20	4	0.38	-0.17	1.60
Duration (year)	< 1	25	1.18	0.37	2.36
	1 - 3	114	0.05	-0.06	0.18
	> 3	7	0.26	-0.15	0.83

**Table S2.3** Weighted response ratio ( $\ln RR_{++}$ ) of soil  $\text{NO}_3^-$  leaching for correlative variables with 95% bootstrap confidence interval (CI).

Variable	Categories	Number	$\ln RR_{++}$	Lower CI	Upper CI
Climate zone	Total	90	-0.14	-0.24	-0.03
	Tropical	5	-0.18	-0.39	0.08
	Temperate	73	-0.11	-0.24	0.03
Land use type	Upland soil	79	-0.14	-0.27	-0.02
	Paddy soil	11	-0.13	-0.26	-0.01
pH	< 5.5	19	0.02	-0.07	0.11
	5.5 -6.5	16	0.03	-0.13	0.23
	6.5 -7.5	23	-0.38	-0.60	-0.13
	> 7.5	24	0.10	-0.02	0.25
Soil texture	Silty clay loam	3	-0.32	-0.53	-0.17
	Loamy sand	33	-0.20	-0.42	0.06
	Sandy loam	4	-0.32	-0.43	-0.19
	Silt loam	10	-0.40	-0.53	-0.22
	Sand	12	0.03	-0.05	0.11
Components	N	54	-0.22	-0.36	-0.07
	NPK	21	0.20	0.03	0.43
Types	Urea	53	-0.15	-0.30	0.01
	$\text{NH}_4\text{NO}_3$	18	-0.23	-0.38	-0.07
Times	1	4	-0.58	-0.71	-0.49
	2	28	-0.21	-0.45	0.06
	3	18	-0.05	-0.2	0.09
Residues	Low C/N residues	4	0.08	-0.21	0.47
	Sawdust	4	0.11	-0.33	1.22
	Barley straw	14	-0.05	-0.19	0.08
	Maize straw	9	0.15	-0.05	0.42
	Rye straw	4	-0.19	-0.42	0.06
	Wheat straw	54	-0.23	-0.36	-0.09
Plow (cm)	0	38	-0.05	-0.18	0.08
	0 – 10	24	-0.27	-0.50	0.02
	10 – 20	15	-0.15	-0.43	0.11
Duration (year)	< 1	8	-0.14	-0.30	0.04
	1 - 3	58	-0.15	-0.29	0.01
	> 3	13	0.06	-0.15	0.36

**Table S3.1** Equations of the stepwise multiple regression for the emission of greenhouse gases (GHG) CO<sub>2</sub> and N<sub>2</sub>O in response to the different types of soil (PUS poor silty soil, RUS rich silty soil, RSS rich sandy soil), and HCA (wheat straw, sawdust, leonardite). The factor P co-application was not significant at this level. Only significant factors are displayed and marked by \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .  $R^2$  adjusted excludes the variance explained by chance.

GHG	Multiple regression equation	$R^2$	$R^2_{\text{adjusted}}$
CO <sub>2</sub>	-0.27 *** + 0.20 Soil*** - 0.01 Time*** + 0.11 HCA***	0.440	0.438
N <sub>2</sub> O	-13.15 * + 15.23 Soil*** - 0.86 Time*** + 3.28 HCA*	0.115	0.112

**Table S3.2** Equations of the stepwise multiple regression for the greenhouse gases emission rate (GHG) of CO<sub>2</sub> and N<sub>2</sub>O of PUS (nutrient-poor silty soil), RUS (nutrient-rich silty soil), and RSS (nutrient-rich sandy soil) in response to the different HCA types (wheat straw, sawdust, leonardite), and the P co-application levels (P<sub>0</sub>, P<sub>140</sub>, P<sub>250</sub>). Only significant factors are displayed and marked by \**P*<0.05, \*\**P*< 0.01, \*\*\**P*<0.001. R<sup>2</sup> adjusted excludes the variance explained by chance.

Soil	GHG	Multiple regression equation	<i>R</i> <sup>2</sup>	<i>R</i> <sup>2</sup> <sub>adjusted</sub>
PUS	CO <sub>2</sub>	0.00635 *** + 0.00101 HCA* + 0.00003 P***	0.081	0.075
	N <sub>2</sub> O	0.00939 *** - 0.00015 Time*** - 0.00044 HCA*	0.242	0.236
RUS	CO <sub>2</sub>	0.33 *** -0.01 Time*** + 0.12 HCA***	0.359	0.354
	N <sub>2</sub> O	37.22 ** -2.18 Time*** + 10.14 HCA*	0.140	0.134
RSS	CO <sub>2</sub>	0.07* - 0.01 Time*** + 0.22 HCA***	0.673	0.671
	N <sub>2</sub> O	13.91*** - 0.40 Time***	0.134	0.131

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**Table S3.3** The detailed information about the pH value and heavy metal contents of leonardite

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	As	Pb	Cd	Cu	Ni	Hg	Zn	Cr	B	Mn	pH
Content (mg/kg)	0.8	2.0	0.2	1.0	1.0	0.07	1.0	1.0	1.0	1.0	4.2

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**Table S5.1** Dates and amounts of compost and fertilizer application during the lysimeter experiment.

Period	Treatment	19 Oct.2019	17 Mar. 2020		15 Apr. 2020	11 May 2020
		Compost (t ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	P (kg ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	N (kg ha <sup>-1</sup> )
Wheat	P1		45	45	81	35
	P2		45	90	81	35
	CP1	40	45	45	81	35
	CP2	40	45	90	81	35
	CCP1	80	45	45	81	35
Barley			8 Mar. 2020		13 Apr. 2020	4 May 2020
			N	P	P	N
			(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )
	P1		60	60	67.5	67.5
	P2		60	60	67.5 60	67.5
	CP1		60	60	67.5	67.5
	CP2		60	60	67.5 60	67.5
	CCP1		60	60	67.5	67.5

Yara: (YaraMila Universal) was applied in the first fertilization.

CAN: (calcium ammonium nitrate) was applied as the subsequent N fertilization.

TSP: (triple superphosphate) was applied as the extra P fertilization.



**Table S5.2** The cumulative emissions of CO<sub>2</sub>-C, CH<sub>4</sub>-C, N<sub>2</sub>O-N during the experiment. No significant difference between each treatment ( $P > 0.05$ ).

Period	HCA	CO <sub>2</sub> -C (t ha <sup>-1</sup> )	CH <sub>4</sub> -C (kg ha <sup>-1</sup> )	N <sub>2</sub> O-N (kg ha <sup>-1</sup> )
Wheat growing season	P1	3.053 ± 0.569	-0.513 ± 0.267	0.141 ± 0.282
	P2	3.013 ± 0.607	-0.730 ± 0.686	0.068 ± 0.096
	CP1	4.447 ± 1.351	-0.139 ± 0.228	0.072 ± 0.144
	CP2	5.457 ± 1.322	-0.471 ± 0.282	0.203 ± 0.159
	CCP1	3.495 ± 0.748	-0.496 ± 0.426	0.610 ± 1.056
	Bare	16.456 ± 3.888	-0.352 ± 0.137	1.979 ± 2.288
Barley growing season	P1	16.173 ± 3.611	-0.203 ± 0.406	1.088 ± 0.887
	P2	11.121 ± 2.583	0.029 ± 0.143	0.613 ± 0.281
	CP1	11.323 ± 4.429	-0.066 ± 0.132	1.085 ± 0.672
	CP2	16.207 ± 5.216	-0.293 ± 0.291	1.607 ± 0.832
	CCP1	20.765 ± 6.879	-0.514 ± 0.518	2.140 ± 1.527

**Table S5.3** Equations of the linear regressions for the soil DOC, P-CAL, and CO<sub>2</sub>-C flux in response to time (T) (days since the beginning of the wheat or barley growing season), compost (C) (t ha<sup>-1</sup>), and phosphorus (P) (kg ha<sup>-1</sup>) fertilization. \**P*<0.05, \*\**P*< 0.01, \*\*\**P*<0.001.

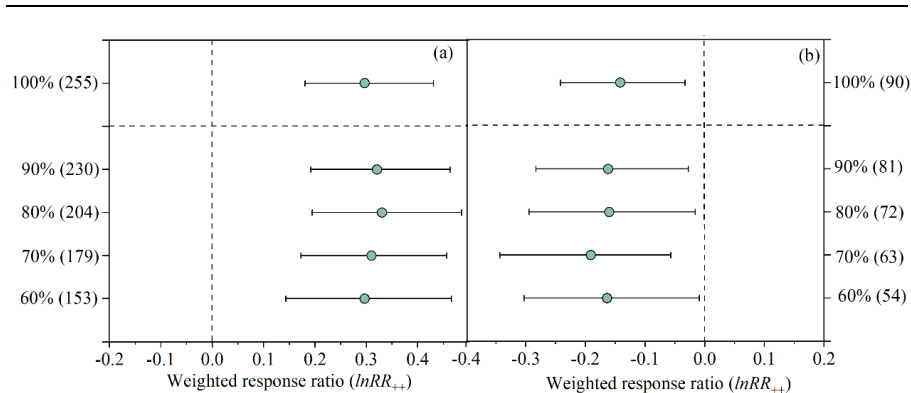
Category	Linear regression equation
DOC	12.869*** – 0.826T*** – 0.003P + 0.084C***
P-CAL	12.673** + 0.071T + 0.014P + 0.317C***
SOC	3.349** + 0.264T – 0.005P + 0.062C***
TN	0.345** + 0.33T* – 0.001P + 0.005C***
TP	0.242*** + 0.038T*** + 0.001P + 0.001C***
CO <sub>2</sub> -C (wheat season)	20.236 + 9.333T*** – 1.007P + 1.546C**
CO <sub>2</sub> -C (barley season)	– 358.981*** + 19.554T*** – 1.590P* + 0.001C

**Table S5.4** Equations of the linear regressions for the crop aboveground biomass and grain yield in response to compost (C) (t ha<sup>-1</sup>), phosphorus (P) (kg ha<sup>-1</sup>) fertilization. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .

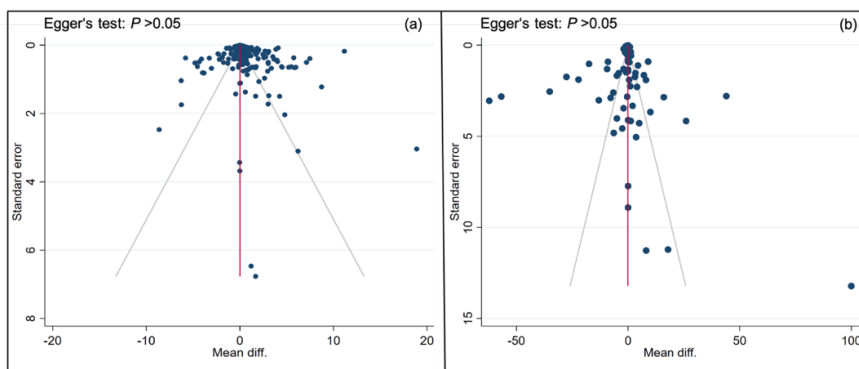
Crop	Soil properties	Linear regression equation
Wheat	Aboveground biomass	$118.879^{***} + 0.008P + 0.821C^{***}$
	Grain yield	$41.924^{***} + 0.107P + 0.311C^{**}$
Barley	Aboveground biomass	$118.609^{***} + 0.070P + 0.385C^{*}$
	Grain yield	$79.011^{***} + 0.037P + 0.278C^{*}$



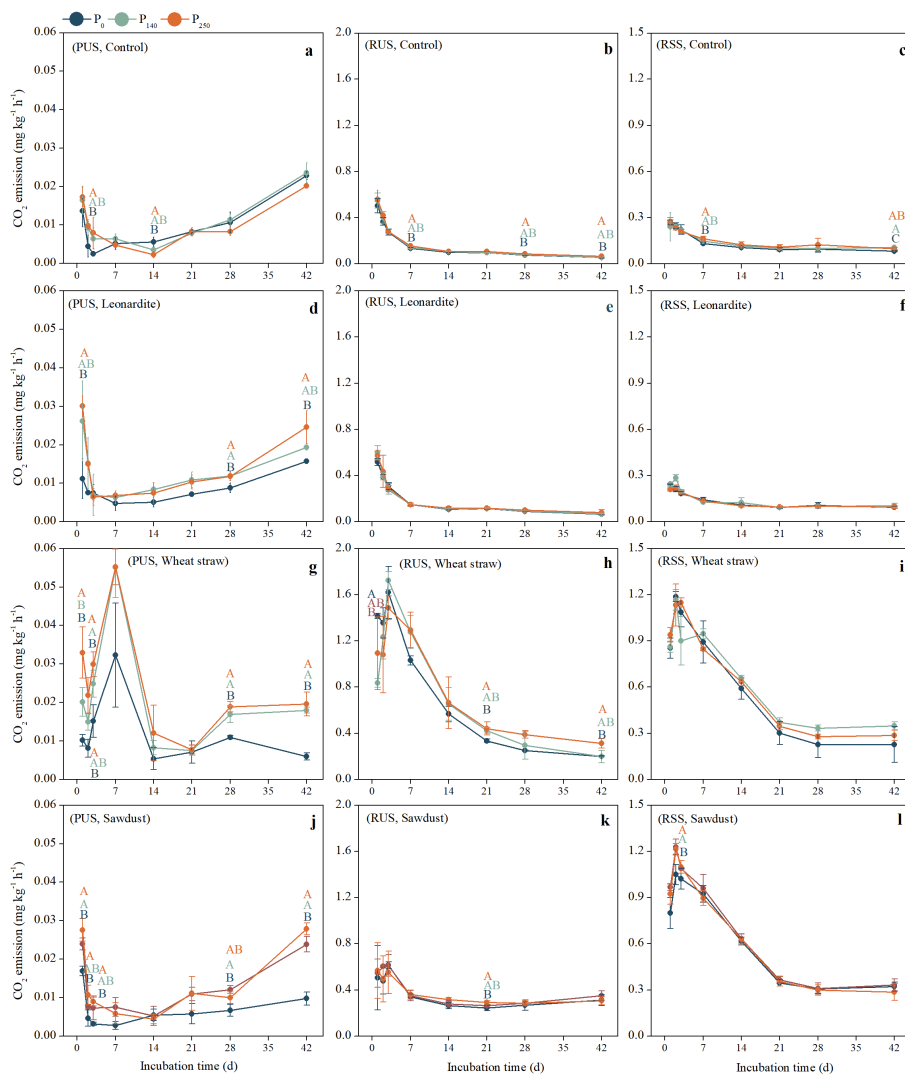
**Figure S2.1** Global map of study sites that provided data of soil  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching for the meta-analysis



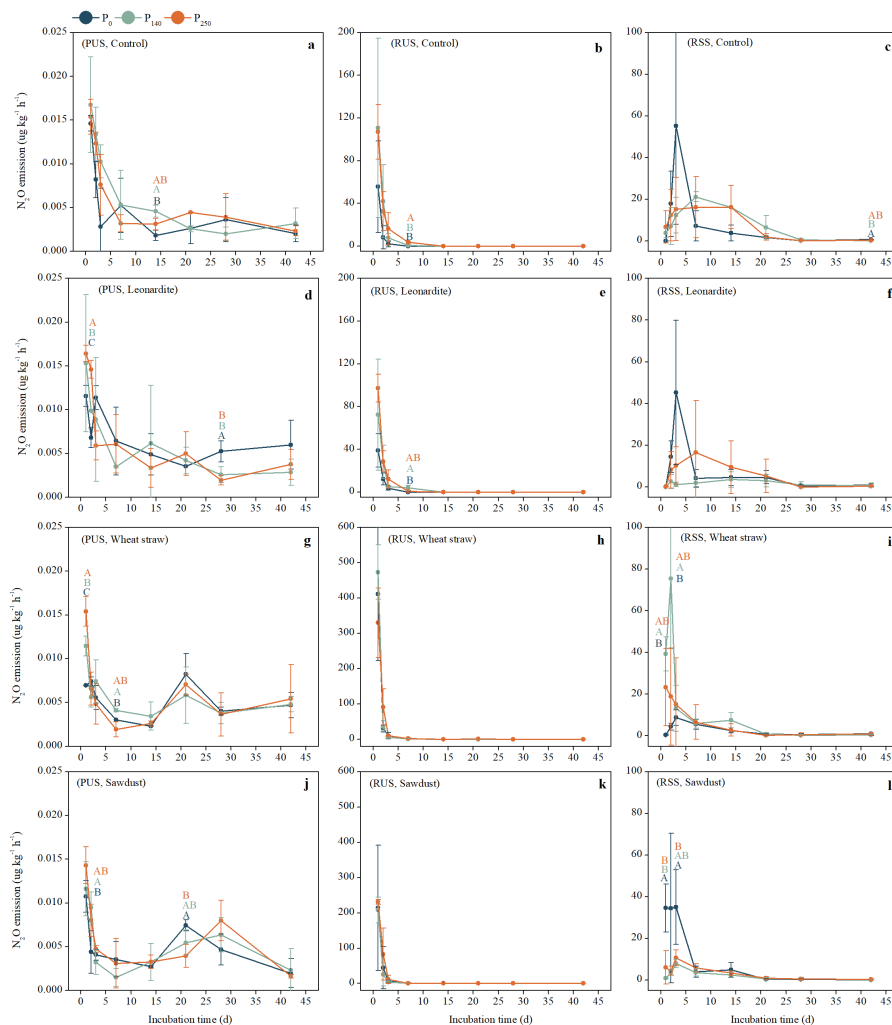
**Figure S2.2** Repeated analysis of the effects of residue return on N<sub>2</sub>O emission (a) and NO<sub>3</sub><sup>-</sup> leaching (b) for different sample sizes.



**Figure S2.3** Funnel plot for estimates in meta-analysis of the effect of residue return on  $\text{N}_2\text{O}$  emission (a) and  $\text{NO}_3^-$  leaching (b).



**Figure S3.1** Mean values and standard deviation of CO<sub>2</sub> emission rates during 42 days of incubation for the control treatment, the treatments with different types of HCA (wheat straw, sawdust, leonardite), and P co-application levels (P<sub>0</sub>, P<sub>140</sub>, P<sub>250</sub>) in PUS (poor silty soil), RUS (rich silty soil), and RSS (rich sandy soil). Different uppercase letters denote significant differences between the P co-application levels within the same treatment (control or type of HCA) at  $P < 0.05$ .



**Figure S3.2** Mean values and standard deviation of  $N_2O$  emission rates during 42 days of incubation for the control treatment, the treatments with different types of HCA (wheat straw, sawdust, leonardite), and P co-application levels ( $P_0$ ,  $P_{140}$ ,  $P_{250}$ ) in PUS (poor silty soil), RUS (rich silty soil), and RSS (rich sandy soil). Different uppercase letters denote significant differences between the P co-application levels within the same treatment (control or type of HCA) at  $P < 0.05$ .





**Figure S5.1** Filling of the lysimeters with intact soil (left) and the lysimeter installation on the campus of Forschungszentrum Jülich (right).

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