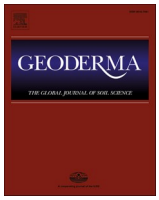
Geoderma 461 (2025) 117500

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma



High nitrogen retention potential of cellulose and starch applied to four soils under simulated post-harvest conditions

Kerui Zhao [a,b,*], Rüdiger Reichel [a], Holger Wissel [a], Xiao Lu [a], Nicolas Brüggemann [a,b]

[a] Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, Agrosphere (IBG-3), Jülich, Germany
[b] Institute of Crop Science and Resource Conservation (INRES), Soil Science and Soil Ecology, University of Bonn, Bonn, Germany

ARTICLE INFO

Handling Editor: Daniel Said-Pullicino

Keywords:
Amino acids
High-carbon soil amendments
Mitigation of nitrogen loss
Microbial nitrogen immobilization
Post-harvest condition
Soil nitrogen fractions

ABSTRACT

Conventional agriculture often leaves surplus mineral nitrogen (N_{min}) after harvest, increasing the risk of nitrogen (N) loss. Applying high soil carbon amendments (HCA) can promote microbial N retention when catch crops are not feasible. However, the effects of soil type on microbial N immobilization in response to HCA application are insufficiently understood. Here, we applied 50 kg N ha^{-1} as ^{15}N-labelled $(NH_4)_2SO_4$ and 4 t C ha^{-1} as starch or cellulose to the Ap horizon of four contrasting agricultural soils in different soil organic carbon (SOC) and texture: alkaline silty Luvisol (SOC 0.93 %) and Regosol (SOC 0.35 %), and acidic sandy Luvisol (SOC 0.58 %) and Gleysol (SOC 1.72 %). Soils were incubated for 98 days at 8.6 °C and 65 % water-holding capacity, simulating post-harvest conditions in North Rhine-Westphalia, Germany. After 98 days under post-harvest condition, cellulose application reduced N_{min} by 50–140 kg N ha^{-1} depending on the soil type, while starch induced faster N_{min} retention, followed by gradual re-release of N_{min} towards the end of the incubation period. HCA-amended soil showed that most of the ^{15}N was recovered in the soil N pool inaccessible to multiple extractions (N_{ret}), rather than in microbial biomass. In HCA-amended clay soils, the recovery of ^{15}N in N_{ret} was approximately twice that of the Control. In sandy acidic soils, HCA increased the recovery of ^{15}N in N_{ret} to levels comparable to those in alkaline soils, while in SOC-rich sandy loam soils, only cellulose caused a slight increase in N_{ret}. After incubation, up to 41 % of labeled N_{min} was stabilized in the form of amino acids, with a more pronounced effect when HCA was added. We conclude that labile organic C can promote the conversion of excess N_{min} into more stable N forms, such as amino acids or peptides, that are able to stabilize N beyond the post-harvest period.

1. Introduction

Excessive nitrogen (N) fertilizer application and mineralization of crop residues and soil organic matter (SOM) lead to the accumulation of mineral N (N_{min}) in agricultural soils, especially in the post-harvest period, which in turn leads to a number of different forms of N loss (Robertson & Vitousek, 2009; X. Zhang et al., 2015). This not only puts pressure on the environment, but also reduces the profitability of farming operations (Kanter et al., 2015). Since N_{min} is more soluble and reactive than organic N forms, it plays a key role in N leaching and gaseous N emissions (Qiu et al., 2024). Unlike ammonium (NH_4^+), which can be partially fixed by the soil matrix, nitrate (NO_3^-), the primary product of the widespread nitrification process in agricultural soils, does not bind to the soil matrix. Consequently, it becomes the major component of the leached N_{min} (Beeckman et al., 2018).

It has been reported that approximately 27 % of groundwater monitoring sites in Europe have NO_3^- concentrations above 50 mg NO_3^- L^{-1}, which is five times higher than EU-Nitrate Directive threshold for drinking water, predominantly observed on agricultural land (Biernat et al., 2020; BMEL & BMU, 2020; Kühling et al., 2021; Sundermann et al., 2020). To reduce N losses, considerable attention has been paid to crop N management during the growing season to minimize the imbalance between crop N demand and soil N accumulation (Chikowo et al., 2004; Shang et al., 2024). However, even without over fertilization, soil samples have been found to contain up to 100 kg ha^{-1} of N_{min} after harvest (Henke et al., 2008). The conventional practice of ploughing to a depth of 20–30 cm after harvest has been demonstrated to further accelerate the mineralization of SOM, leading to additional N_{min} accumulation (Koch & Stockfisch, 2006). Moreover, the excess N_{min} is susceptible to leaching due to the slow growth and low N uptake rates of

* Corresponding author.
E-mail address: k.zhao@fz-juelich.de (K. Zhao).

https://doi.org/10.1016/j.geoderma.2025.117500
Received 29 April 2025; Received in revised form 27 August 2025; Accepted 1 September 2025
Available online 3 September 2025

winter crops planted in autumn or early winter (Scheller & Joergensen, 2008). It is therefore essential to facilitate the conversion of the remaining N_{min} into more stable N forms, particularly during the post-harvest period (Power & Peterson, 1998; Sieling & Kage, 2006).

Despite the existence of studies that have investigated the uptake of excess N_{min} by catch crops during winter, the ultimate effect of N_{min} reduction still varies depending on climatic conditions, crop rotation management and the characteristics of the catch crop species (Teixeira et al., 2016; Thapa et al., 2018). The stimulation of N_{min} incorporation into microbial biomass and its subsequent conversion to organic N forms may have an effect comparable to that observed in catch crops (Neal et al., 2023). In general, microorganisms continuously assimilate N from the soil, incorporating it into microbial biomass or extracellular polymeric substances (EPS) through metabolic processes and turnover (Liang et al., 2010). It is important to note, however, that microbial N assimilation is not only affected by the N supply, but also by soil carbon (C) availability. An excess of available N results in a relative C deficiency, which in turn promotes SOM mineralization and a further release of N_{min} (Jansson & Persson, 2015; Manzoni et al., 2012; Said-Pullicino et al., 2014; Smith et al., 2008). Therefore, replenishing agricultural soils with appropriate high-carbon soil amendments (HCA) represents an effective strategy for the reduction of SOM degradation and the retention of N within the soil. This approach serves to minimize N loss to the environment while simultaneously enhancing N use efficiency (NUE) (Elrys et al., 2022).

Cellulose and starch are the most abundant C sources on Earth and therefore have significant potential as HCA (Kim & Dale, 2004; MacNeill et al., 2017; Saka & Ueno, 1999). Cellulose is primarily found in plant biomass and remains in large quantities on agricultural fields after harvest in the form of crop residues, with an estimated 3.8 billion tons of crop residues produced annually (Liang et al., 2010). Similarly, starch is considered to be the second most abundant form of biomass (Villa Zabala & Villa Zabala, 2020). Both cellulose and starch are N-free glucose polymers, that exhibit different characteristics with respect to their structural composition and degradation pathways. Starch consists of glucose linked by α-1,4-glycosidic bonds and branched by α-1,6-glycosidic bonds, which facilitate its degradation by amylase into the monomer glucose, which can then be readily utilized by microbes. In contrast, cellulose is a linear polymer composed of β-1,4-linked D-glucose, which requires a greater investment of enzymatic resources for degradation (Allison et al., 2014; Fanin et al., 2020; Wang et al., 2020). This structural difference indicates that cellulose is more resistant to microbial decomposition than starch. Previous research on litter decomposition and soil C cycling, using both cellulose and starch, has shown that nitrogen-free HCA ($HCA_{N\text{-}free}$) can initiate microbial decomposition and alter the native soil C pools within a few days (Rabbi et al., 2024; Wilhelm et al., 2022; Xing et al., 2023; Zhao et al., 2025). However, it is unclear how effective the application of these two $HCA_{N\text{-}free}$ is in immobilizing excess N_{min} under post-harvest conditions.

In addition, the type of immobilized N pool stimulated by $HCA_{N\text{-}free}$ is not yet clear. Previous research indicated that the assimilation of N_{min} into microbial biomass N (N_{mic}) serves as the principal soil reservoir for stored N (Burger & Jackson, 2003; Johnson et al., 2000). However, the N present within living microbial biomass accounts for only 5–10 % of the soil total nitrogen (N_{tot}) (Deng & Liang, 2022). In contrast, the remaining necromass after microbial death, including proteins, chitin, peptidoglycan and nucleic acids, account for over 60 % of N_{tot} (Liang et al., 2017; Wang et al., 2021). In addition, in contrast to the high mobility and solubility of N_{min}, the N-rich microbial products, varying in size from monomers to macromolecules, can form highly stable soil organic matter (SOM) through interactions with functional groups of organic molecules associated to soil mineral surfaces (Jilling et al., 2018; Lehmann & Kleber, 2015).

Amino acids, fundamental units in protein formation, are more abundant and exhibit a faster turnover rate than other N-containing organic substances. Consequently, their contribution to organic matter production is more than twice that of other N-containing organic substances (Hu et al., 2020; Kuzyakov, 1997). Furthermore, studies have found that crops can acquire amino acids, either directly through root cell membranes or via mycorrhizal fungi, which further underscores the need for amino acids to be included in soil N management (Liu et al., 2024; Pan et al., 2022). While a significant number of studies have focused on the products of microbial metabolic processes, only a limited number focused on the role of amino acids in regulating N_{min} during agricultural N immobilization (Xu et al., 2024). It is therefore essential to quantify the contribution of amino acids as a relatively stable N pool for immobilized N_{min} and to assess the stability of newly synthesized amino acids.

However, soils vary considerably in their properties, particularly in terms of pH, texture, and SOM content, which may have a significant influence on the retention of microbial-derived N under post-harvest conditions. It is widely acknowledged that acidic soils have an inhibitory effect on microbial activity, resulting in a reduction in microbial growth and activity (Malik et al., 2018; Tripathi et al., 2018). However, some studies have suggested that a moderately acidic pH may facilitate the adsorption of N compounds to minerals (Hwang & Lenhart, 2008; Parikh et al., 2011; Yu et al., 2013). A higher clay content typically facilitates the stabilization of organic matter through the formation of robust organo-mineral associations (Chenu et al., 2000; Islam et al., 2022; Xu et al., 2016). The soil organic carbon (SOC) content of soil defines the baseline level of microbial biomass that can be maintained in a given soil over time. This is a crucial factor that can be modified by HCA (Craig et al., 2021; Six et al., 2002), and this highlights the importance of understanding how fundamental soil properties affect microbial process, particularly with regard to microbial-mediated N retention in the post-harvest phase. However, there is still a paucity of information in the literature on the latter and the role of readily accessible C in the microbial production of organic N forms for long-term soil N storage (Clocchiatti et al., 2023).

To trace the fate of fertilizer-derived N in different N pools and to investigate the consequences of N immobilization in agricultural soils under post-harvest conditions, the present study employed a ^{15}N stable isotope labeling approach with four different soils, using cellulose and starch as $HCA_{N\text{-}free}$. We conducted a fully controlled 98-day soil incubation experiment under realistic post-harvest temperature and moisture conditions. The objectives were: (1) to assess the magnitude and timing of N_{min} retention induced by the two $HCA_{H\text{-}free}$ under post-harvest conditions; (2) to investigate the impact of $HCA_{H\text{-}free}$ and soil properties on the formation and stability of the newly synthesized immobilized N pool by using the soil N fractionation method; (3) to identify the role of amino acid compounds in the immobilization of fertilizer-derived N_{min}.

2. Material and methods

2.1. Simulation of the post-harvest phase

The present study focused on the post-harvest period, a critical time for N losses, defined as the period between seedbed preparation for winter sowing and the onset of the next growing season. This period typically lies between October and April in the temperate climate of the northern hemisphere, as this period comprises most precipitation days, and N uptake of winter crops is insufficient to offset N_{min} accumulation in soil. The average monthly temperature was based on data from a weather station at Maastricht Aachen Airport between 2015 and 2019, with an average monthly temperature during the post-harvest period of 8.6 °C. Soil moisture content varied between October and April of next year, with a trend of 60 %–80 %–60 % water holding capacity (WHC) (Zhao et al., 2025). Our incubation study used the average soil moisture of the post-harvest period, which was 65 % WHC.

2.2. Soil substrates

Four soils were collected from different sites in Germany. The first soil (CKA) was obtained from the upper 20 cm of farmland at the agricultural research station Campus Klein-Altendorf (50.615424° N, 6.992823° E) of the University of Bonn, Germany. The soil was classified as a Haplic Luvisol. In accordance with standard agricultural practice, the sampling area was limed eight months prior to sampling. The second soil (INDEN) was sampled from the upper 20 cm in a recultivation area of an opencast mining site (50.882444° N, 6.373207° E), Inden, Germany. The soil texture was classified as a silty loam and the soil type as a Calcaric Regosol (Pihlap et al., 2019). Furthermore, two sandy soils were obtained from the Landwirtschaftliche Untersuchungs und Forschungsanstalt (LUFA) in Speyer, Germany. The third soil (LUFA 2.1) soil was classified as a Luvisol, sampled from Dudenhofen, Germany (49.318476° N, 8.383514° E). The fourth soil (LUFA 2.2) was a Mollic Fluvic Gleysol, sampled from Hanhofen, Germany (49.312629° N, 8.327040° E). The fresh soil was sieved at 2 mm and homogenized prior to storage at room temperature. Soil organic carbon (SOC) was determined using an elemental analyzer (vario EL Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) and a multi-phase carbon/hydrogen/water analyzer (RC612, LECO Instrumente GmbH, Mönchengladbach, Germany). Due to the high inorganic carbon content, CKA and INDEN soils were pretreated with an acid solution to remove inorganic C. Air-dried subsamples from four soils were analyzed for SOC, which ranged from 0.35 to 1.72 %. For analysis of WHC, extractable N_{min} and pH, the detailed methods were the same as described in Sections 2.4.1 and 2.4.2. A complete digestion of 50 mg air-dry soil was carried out with lithium borate melt to determine the content of Fe, Al and Ca with ICP-OES. All basic soil properties are given in Table 1.

2.3. Experimental setup

To simulate a typical amount of excess N_{min} at the beginning of the post-harvest period, the equivalent of 50 kg N ha^{-1} was set as the amount of N_{min} accumulated in the soils. To trace the fate of excess N_{min}, highly ^{15}N-enriched ammonium sulfate ($(NH_4)_2SO_4$ with 98 atom% ^{15}N, VWR, Germany) was diluted to 2.96 atom% ^{15}N by mixing it with $(NH_4)_2SO_4$ with a natural ^{15}N abundance of 0.367 atom% ^{15}N (VWR, Germany). Each HCA treatment received the equivalent of 4 t C ha^{-1}, applied to the 0–30 cm layer of the topsoil, which corresponds to a value close to the maximum amount of wheat straw available per hectare after harvest (average 7–8 t wheat straw biomass per hectare) (Montero et al., 2018). The effects of HCA application were determined using the two HCA cellulose (Cellulose) and starch (Starch) separately, as well as a 50:50 mixture of cellulose and starch (Cellulose + Starch). Cellulose and starch were purchased from Sigma Aldrich (Germany), with 44.4 % of C and zero N. In addition, a treatment without addition of HCA and N (Blank) and a treatment with N addition only (Control) were incubated in parallel under the same conditions.

To create a homogeneous soil matrix, 498 g fresh soil, 26.4 mg ammonium sulfate [$(NH_4)_2SO_4$] (equivalent to 11.11 mg N kg^{-1} soil or 50 kg N ha^{-1} in the 0–30 cm layer), and the respective HCA, containing 445 mg C, were homogeneously mixed in a big bowl and then filled into polypropylene (PP) sewage tubes (5 cm diameter, 30 cm length, HT-Rohr, Marley Deutschland GmbH, Germany). The tubes were sealed at the bottom and filled up to 5 cm from the top, while the soil was compacted to field bulk density (1.5 g cm^{-3}). The total number of soil columns was 400, including four soils, five treatments, four replicates and five sampling times. To avoid the effect of differences in background NO_3^- in the different soil substrates, an extra 12.6, 15.8 and 5.4 mg N kg^{-1} unlabeled NO_3^- was added to the INDEN, LUFA 2.1 and LUFA 2.2 soils, respectively, to reach a similar NO_3^- content as in the CKA soil.

To ensure sufficient recovery of microbial activity, the soil water content was increased to 40 % WHC over 24 h at 8.6 °C, and then to the desired 65 % WHC on day of the experiment (DoE) 0, the start of the post-harvest incubation. All soil tubes were placed in incubators (TS 608/2i, Xylem Analytics GmbH, Weilheim, Germany) to incubate the soil tubes at 8.6 °C. The soil tubes were randomly placed and their positions rotated periodically in the incubators to minimize bias from potential temperature heterogeneities in the incubators. Soil moisture was adjusted twice a week based on weight loss.

Table 1
Initial pH, organic C, mineral N (NH_4^+ and NO_3^-), iron (Fe), aluminum (Al) and calcium (Ca) contents of the four soils used in the study.

	CKA	INDEN	LUFA2.1	LUFA2.2
Soil type	Haplic Luvisol	Calcaric Regosol	Luvisol	Mollic Fluvic Gleysol
Soil texture	Silt loam	Silt loam	Sandy	Sandy loam
Clay (%)	15	16	3.5	10.8
Silt (%)	77	79	9.6	16.9
Sand (%)	8	5	87	72
pH (0.01 M $CaCl_2$)	8.0	7.7	4.9	5.4
Organic C (%)	0.93	0.35	0.58	1.72
WHC (%)	32.3	31.7	19.9	32.1
NH_4^+ (mg N kg^{-1})	0.7	0.3	3.4	1.1
NO_3^- (mg N kg^{-1})	23.0	10.3	7.2	17.6
Fe (%)	2.13	2.17	0.46	0.50
Al (%)	3.91	4.2	1.74	2.32
Ca (%)	0.86	3.07	0.09	0.19

Origin of the four soils: "CKA" means Campus of Klein-Altendorf, "INDEN" means Inden recultivated area, "LUFA 2.1" and "LUFA 2.2" were obtained from LUFA Speyer, Germany. "WHC" means maximum water holding capacity of soils.

2.4. Soil sampling and analyses

The entire experiment involved five soil samplings, taken at DoE 0, 14, 28, 56 and 98. Four soil tubes were collected for each treatment during each soil sampling. Soil was sampled from the entire PP tube using a long-shafted stainless-steel spoon. The soil was mixed homogeneously in a large bowl. The mixed samples were then sealed in plastic bags and stored at –22 °C until further processing. Before extracting and fumigating the soil samples in the laboratory, the soil was removed from the freezer and thawed at room temperature for 30–40 min.

2.4.1. Determination of WHC and soil pH

The determination of the WHC of the soil followed the procedure described by Schinner et al. (2012). Four plastic rings, each with a volume of 100 cm^3, were filled with soil at a soil bulk density of 1.5 g cm^{-1}, and placed on a sand bath The cylinders were immersed in water, with the water level adjusted to the height of the soil cylinders. After 15 h, the excess water was drained over 3 h to allow for equilibration. The saturated soils were transferred into pre-weighed porcelain dishes. The samples were dried at 105 °C for 24 h until they reached a constant weight, after which they were weighed again. The WHC was then calculated from the difference between the saturated and dry weights, and the average value was calculated. The soil pH was determined in the solution extracted with 0.01 M $CaCl_2$ in a 1:4 soil/solution ratio using a pH meter (multi 340i, WTW GmbH, Weilheim, Germany) according to the ISO 10390 method. The pH was determined for soil samples collected at DoE 0, 14, 28, 56, 98.

2.4.2. Soil N extraction and analysis

Based on the different extraction capacities of N_{min} by 0.01 M $CaCl_2$ and 1 M KCl (Kachurina et al., 2000), a sequential extraction was performed to determine N_{min}, exchangeable N_{min} and the retained organic N (N_{ret}) pool. For N_{min} analysis, including NH_4^+, NO_3^- and NO_2^-, 10 g of

fresh soil was extracted with 40 ml 0.01 M $CaCl_2$, shaken at 200 rpm for 2 h, centrifuged with 2876 relative centrifugal force (RCF) for 25 min, and filtered using 0.45-μm PP-membrane syringe filters (VWR International, Darmstadt, Germany). The filtrate was analyzed via continuous flow analysis and ion chromatography (Dionex DX-500, Thermo-Scientific, USA), as described by Houba et al. (2000). The N_{min} content was determined by adding the contents of NH_4^+, NO_3^- and NO_2^-. After extraction of N_{min}, the soil pellet remaining after centrifugation was extracted with 1 M KCl to extract the exchangeable N_{min} (Chen et al., 2024b). The soil pellet was re-suspended with 40 ml 1 M KCl and shaken at 200 rpm for 1 h, then filtered and processed as described above. The soil residue was then washed with 40 ml deionized water and shaken by hand until the soil particles were completely re-dispersed. The samples were then centrifuged at 2876 RCF for 25 min. The supernatant was discarded, and the soil pellet was finally dried at 60 °C for 24 h. After oven drying, 5 g of dry soil were ball-milled for 2 min (MM 2, Retsch GmbH, Haan, Germany). Finally, 40 mg of the ground soil was placed in tin capsules (5 mm x 9 mm, IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany) for the determination of N_{ret} and ^{15}N isotope signature using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Flash EA coupled to Delta V plus, Thermo Fisher Scientific, Bremen, Germany).

2.4.3. Soil microbial biomass carbon and nitrogen

Soil microbial biomass C (C_{mic}) and N_{mic} were extracted using the chloroform fumigation-extraction method (Joergensen et al., 1995; Reichel et al., 2017). Briefly, each soil sample was divided into two parts of 10 g fresh weight each, one part was filled into a 50-ml glass jar and fumigated with ethanol-free chloroform in a desiccator for 24 h. The other part of the soil sample was subjected to extraction without prior fumigation. Both fumigated and non-fumigated soil samples were extracted with 40 ml of 0.01 M $CaCl_2$, and the extraction method was the same as the N_{min} extraction method described above. Dissolved organic C (DOC) and total dissolved N (DN) were determined using a TOC/TN analyzer (TOC-VcPH + TNM-1 + ASI-V, Shimadzu, Japan), both before and after chloroform fumigation, to quantify C_{mic} and N_{mic}. Values of C_{mic} and N_{mic} were calculated using the correction factors kEC 0.45 and kEN 0.40, respectively (Joergensen & Mueller, 1996).

2.4.4. ^{15}N tracing analyses

To follow the fate of the ^{15}N-labeled N_{min} added to the soil before the start of the incubation, the ^{15}N recovery rates in different soil N pools were determined. The ^{15}N enrichment of N_{min} was determined in an aliquot of the mineral N extract (see 2.4.1). For this purpose, 10 ml of the 0.01 M $CaCl_2$ extract was freeze-dried. The solid was then re-dissolved in 200 μl of deionized water and transferred to tin capsules (5 mm x 12 mm, IVA Analysentechnik). After air drying for 96 h at room temperature in a fume hood, the tin capsules were packaged and analyzed in the same way as the ball-milled soil samples. Different $(NH_4)_2SO_4$ (^{15}N atom%: 0.367–0.384), KNO_3 (^{15}N atom%: 0.439–0.729), and urea (^{15}N atom%: 0.947) standards were used for ^{15}N calibration. The N content and ^{15}N analysis were determined by EA-IRMS.

After the sequential extraction, ^{15}N enrichment in N_{ret} was measured for each soil sample. For this, 5 g of the extracted soil samples were air dried and ground to powder using a ball mill (see details above). The soil powder was weighed into 5 mm x 9 mm tin capsules (IVA Analysentechnik). The ball-milled soil and dissolved ^{15}N samples were analyzed for total N content and ^{15}N isotope signature using the EA-IRMS as described above. The same standards were used for calibration as described above. The ^{15}N recoveries (%) in the dissolved N, N_{mic} and N_{ret} were calculated using the following equation (Fry, 2006):

$$^{15}N_{recovery} = \frac{N_{determined}}{N_{appliedisotopefertilizer}} \times \frac{^{15}N_{soilsamples} - ^{15}N_{background}}{^{15}N_{appliedisotpefertilzer} - ^{15}N_{background}} \quad (1)$$

where $N_{determined}$ is the N content of dissolved N in fumigated and non-fumigated soil, and total soil N in soil samples; $N_{applied\ isotope\ fertilizer}$ is the N content of the applied N fertilizer; $^{15}N_{soil\ samples}$ is the ^{15}N content (atom%) in soil samples; $^{15}N_{applied\ isotope\ fertilizer}$ and $^{15}N_{background}$ are the average content of ^{15}N (atom%) of the applied $(NH_4)_2SO_4$ and of non-isotopically labeled N, respectively. ^{15}N recovery in microbial biomass N ($^{15}N_{mic}$) was determined as the difference in $^{15}N_{recovery}$ between fumigated and non-fumigated soil $CaCl_2$ extracts.

2.5. Gas sampling and analysis

Emissions of CO_2 and N_2O were measured at DoE 1, 2, 3, 5 and 7 to capture the rapid dynamics at the beginning of the experiment. From DoE 7 onwards, measurements were performed every week. The PP tubes were taken out of the incubator 2.5 min prior to the gas measurement to allow equilibration with ambient air, before the PP tube was connected to an airtight PP headspace chamber (HT drainpipe coupler, 50 mm diameter, 126 mm long, with two sockets and rubber gaskets, closed at one end with a PP stopper). The total volume, including the headspace, was 0.25–0.28 L. The chamber was fitted with Swagelok tube fittings: a vent tube (1.3 m, 1/8″PTFE) and two ¼″fittings for closed-loop connection to an infrared gas analyzer (G2508, Picarro Inc., USA) via 0.63 m ¼″PTFE tubing. Each measurement lasted 10 min, with the first 2 min excluded due to pressure equilibrium. The soil tubes were immediately returned to the incubator to minimize temperature effects. If $R^2 > 0.81$, the emission flux is considered valid. The slope of the linear regression of the gas concentration increase in the combined headspace of tube and headspace chamber was used to calculate the gas flux rate using equation (2):

$$F = \frac{\frac{\Delta c}{\Delta t} \times V \times T_0 \times M}{m \times T_a \times V_m} \quad (2)$$

where F is the gas emission flux (mg $m^{-2}h^{-1}$); $\frac{\Delta c}{\Delta t}$ is the change in gas concentration in ppmv for CO_2 and ppbv for CH_4, NH_3 and N_2O; V is the headspace volume in L; M is the molar mass of N in N_2O and NH_3, or C in CO_2 or CH_4 in g mol^{-1}, respectively; m is the amount of soil in g dry weight; V_m is the molar volume of an ideal gas (22.414 L mol^{-1}) at 0 °C and 101.325 kPa, corrected for the gas sampling temperature using T_0 (273.15 K) and T_a (ambient temperature during the measurement in K). Cumulative fluxes were calculated over 98 days using the trapezoidal method, averaging fluxes between two sampling events, multiplying by the time interval, and summing up all intervals.

2.6. Extraction of amino acid nitrogen

To remove carbonates and reduce the amount of mineral matter and inorganic salts, 10 g soil sample was suspended in 50 ml of 1 M HCl, followed by shaking at 200 rpm for a duration of 16 h by room temperature. The HCl solution was then removed by centrifugation and washing with water. In the next step, the samples were treated with 50 ml of a 5 M hydrofluoric acid (HF) solution by shaking at 200 rpm for 16 h at room temperature (Cheng, 1975; Cheng et al., 1975). The HF solution was removed again by centrifugation and washing with water. After these acid pre-treatments, the soil samples were freeze-dried.

For soil protein hydrolysis, the dry soil samples were suspended in 30 ml of 6 M HCl and incubated at 110 °C for 6 h (Blattmann et al., 2020; Takano et al., 2010). After centrifugation and separation of the remaining insoluble soil, the acid hydrolysis solution was evaporated at 70 °C until dryness. The samples were then resuspended in 5 ml of water and centrifuged to remove the insoluble parts. The non-amino acid organic compounds in the hydrolysates were removed by solid phase extraction (SPE) using a styrene–divinylbenzene copolymer sorbent (Bond Elute PPL cartridges, Agilent Technologies Inc.) (Dittmar et al., 2008; Li et al., 2016). Isolation and complete desalting of amino acids in hydrolysates was performed by cation-exchange chromatography (AG 50 W-X8, 200-400mesh, Bio-Rad Laboratories). Amino acids were eluted

from the cation-exchange sorbent with 10 % NH_3, evaporated at 70 °C until dryness, redissolved in 5 mL water and finally purified by centrifugation to remove insoluble matter. At the end, the purified amino acid extracts were freeze-dried (Blattmann & Ishikawa, 2020; Dittmar et al., 2008; Li et al., 2016; Takano et al., 2010). The remaining steps were identical to those outlined for the analysis of N_{ret}. The ^{15}N content of amino acid N (AAN) in the samples was determined by EA-IRMS, and the ^{15}N recovery in AAN was calculated according to Eq. (2).

2.7. Calculations and statistical analysis

The results are presented as mean values of four replicates ± standard deviation on a dry soil basis. The normality of the distribution was tested with the Kolmogorov-Smirnov normality test. In instances where the data exhibited a non-normal distribution and/or variance heterogeneity, the recommended pairwise Games-Howell test was employed to identify significant relationships. The significance of the differences between treatments was evaluated through a three-way analysis of variance (ANOVA), testing for the effects of incubation time, soils and the application of HCA. Comparisons between means were performed at the 0.05 significance level using a Tukey test (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA).

To identify potential predictors of N_{min} content and ^{15}N recovery in N_{ret}, a stepwise multiple linear regression (SMLR) model was constructed. The parameters included incubation time, soil properties, extractable soil nutrients, gaseous emissions and retained organic nitrogen. During the regression procedure, the variables were eliminated stepwise to determine a minimum subset of variables with maximum explanatory power and to eliminate the inter-correlation between the explanatory variables. The adjusted R^2 (R^2_{adj}) was the coefficient of determination indicating the percentage of variance in the dependent variable explained by the independent variables of the model. As a final criterion for model selection, the Akaike Information Criterion (AIC) was used, with the lowest AIC value indicating the best model fit.

Additionally, structural equation models (SEMs) were constructed, with the significant variables identified in the SMLRs incorporated therein. The study included all treatments except the control to facilitate the establishment of a conceptual framework. The SEMs were calculated using the 'lavaan' package in R (Lavaan, 2012) on the basis of the bivariate relationships and known soil biochemical mechanisms between soil properties, microbial parameters and soil N parameters. Particular attention was paid to the effects of different biological and chemical factors on N_{min} and N_{ret}. The model structure was repeated until the optimal model with the largest comparative fit index (CFI) value was quantified.

3. Results

3.1. Post-harvest effect of HCAs on soil properties related to C and N cycling

The pH values ranged from 4.9 to 8.0 in the four soils and generally showed a decreasing trend over the period of incubation (Fig. S1). Throughout the entire incubation period, the pH values were influenced by the interaction between the soils, the incubation time, and the addition of $HCA_{N\text{-}free}$ (Table S1, $p < 0.001$).

The highest DOC levels occurred in the Starch treatments on DoE 14, reaching 48.2, 65.5 and 91.5 mg kg^{-1} in the INDEN, CKA, and LUFA 2.1 soils, respectively. In contrast, the DOC peaks in the cellulose treatment occurred later and were lower, with levels of 60.8 mg kg^{-1} in CKA soil, 29.9 mg kg^{-1} in LUFA 2.2 soil, and 31.9 mg kg^{-1} in LUFA 2.1 soils. A significant interaction was identified between the soils with regard to DOC, incubation time, and the addition of $HCA_{N\text{-}free}$ (Table S1, $p < 0.001$; Fig. S2). In addition, the $HCA_{N\text{-}free}$ amendments resulted in a higher DOC concentration compared to the Blank and Control (Table S1, $p < 0.001$).

The N_{min} content, comprising NH_4^+, NO_3^- and NO_2^-, was influenced by the interaction of soils, incubation time and $HCA_{N\text{-}free}$ amendments (Table S1, $p < 0.001$). In general, the NO_2^- content was much smaller than the NH_4^+, NO_3^- content. In the CKA, INDEN and LUFA 2.2 soils, a marked decline was detected in NH_4^+ content across all treatments, with reductions ranging from 79–100 % relative to the initial levels from DoE 0 to 14. In contrast, the LUFA 2.1 soil showed differences between treatments (Fig. 1a): The NH_4^+ contents of the Control and Blank in LUFA 2.1 showed an increasing and then decreasing trend over the incubation period, whereas NH_4^+ contents decreased in the $HCA_{N\text{-}free}$ treatments to the minimum level (1.5–1.8 mg kg^{-1}) at DoE 14 or 28, followed by a subsequent increase. The NO_3^- concentration dynamics varied markedly between treatments (Fig. 1b). The most pronounced increase in NO_3^- concentration was observed in the Control treatment of the CKA soil, reaching 83.1 mg N kg^{-1}, which largely accounted for the overall increase in N_{min} (Fig. S3, Table S2). In the $HCA_{N\text{-}free}$ treatments, however, NO_3^- levels decreased to varying extents depending on the soil type and amendment. Among the $HCA_{N\text{-}free}$ treatments, starch addition led to the most rapid NO_3^- decline between DoE 0 and 28, with levels in INDEN soil dropping to 0.7 mg kg^{-1}. The Cellulose treatment in INDEN soil, on the other hand, exhibited an initial decrease in NO_3^- to 4.2 mg kg^{-1} by DoE 56, maintaining relatively low levels thereafter without increases compared to the Starch treatment (Fig. 1b). At DoE 98, the average N_{min} content in $HCA_{N\text{-}free}$ treatments in the INDEN soil at DoE 98 was 4.6 mg N kg^{-1}, significantly lower than in CKA, LUFA 2.1, and LUFA 2.2 soils (Table S2), which recorded values of 18.6, 21.4, and 21.6 mg N kg^{-1}, respectively. Across all soils, N_{min} concentrations in the Blank and Control treatments remained consistently higher than in the $HCA_{N\text{-}free}$ treatments (Fig. S3, Table. S2, $p < 0.05$).

3.2. Post-harvest effect of HCAs on C and N of the microbial biomass

Compared to the Blank and Control, the addition of $HCA_{N\text{-}free}$ resulted in an increase in microbial biomass, although the effects varied among the different soils (Fig. 2). The addition of starch resulted in C_{mic} contents ranging from 323.3 to 841.3 mg C kg^{-1} in the four soils during the initial 28 days of incubation and being significantly higher than in the other treatments (Fig. 2a). While the Cellulose and Cellulose + Starch mixture treatments also exhibited higher C_{mic} than the Control during the same period, they were only 50–79 % of the level observed in the Starch treatments of the CKA, INDEN and LUFA 2.2 soils, and only 18–38 % of the Starch treatment of the LUFA 2.1 soil. However, the elevated C_{mic} observed in the Starch treatments did not persist throughout the incubation period, returned to the initial levels over time. Similarly, the N_{mic} was higher in the $HCA_{N\text{-}free}$ treatments compared to the Control (Fig. 2b), with a notable increase observed in the CKA soil (Fig. 2b, $p < 0.001$). Nevertheless, the N_{mic} exhibited a notable decline in the Starch treatment in the CKA soil during the subsequent incubation period.

3.3. Post-harvest effects of HCAs on emissions of CO_2 and N_2O

The CO_2 emissions from the four soils reflected the stimulatory effect of the soil property and $HCA_{N\text{-}free}$, although the emission dynamics and cumulative emissions were variable (Fig. 3a, b, Table S2). The $HCA_{N\text{-}free}$ treatments showed a more rapid increase in CO_2 emission compared to the Control and Blank, with peaks occurring between DoE 10 to 17. Notably, starch addition resulted in the highest CO_2 flux, with a value of 732.9 mg C $m^{-2}h^{-1}$, closely followed by the peaks observed in the Cellulose + Starch mixture treatment. The increased fluxes induced by the $HCA_{N\text{-}free}$ resulted in higher cumulative CO_2 emission compared to Control and Blank ($p < 0.05$), although the magnitude differed between soils. While the starch application resulted in the highest cumulative CO_2 emission, the Cellulose treatment exhibited delayed emission peaks with lower CO_2 fluxes, resulting in cumulative emissions that were 10.4 to 68.1 g C m^{-2} lower than those of the Starch treatment across the four

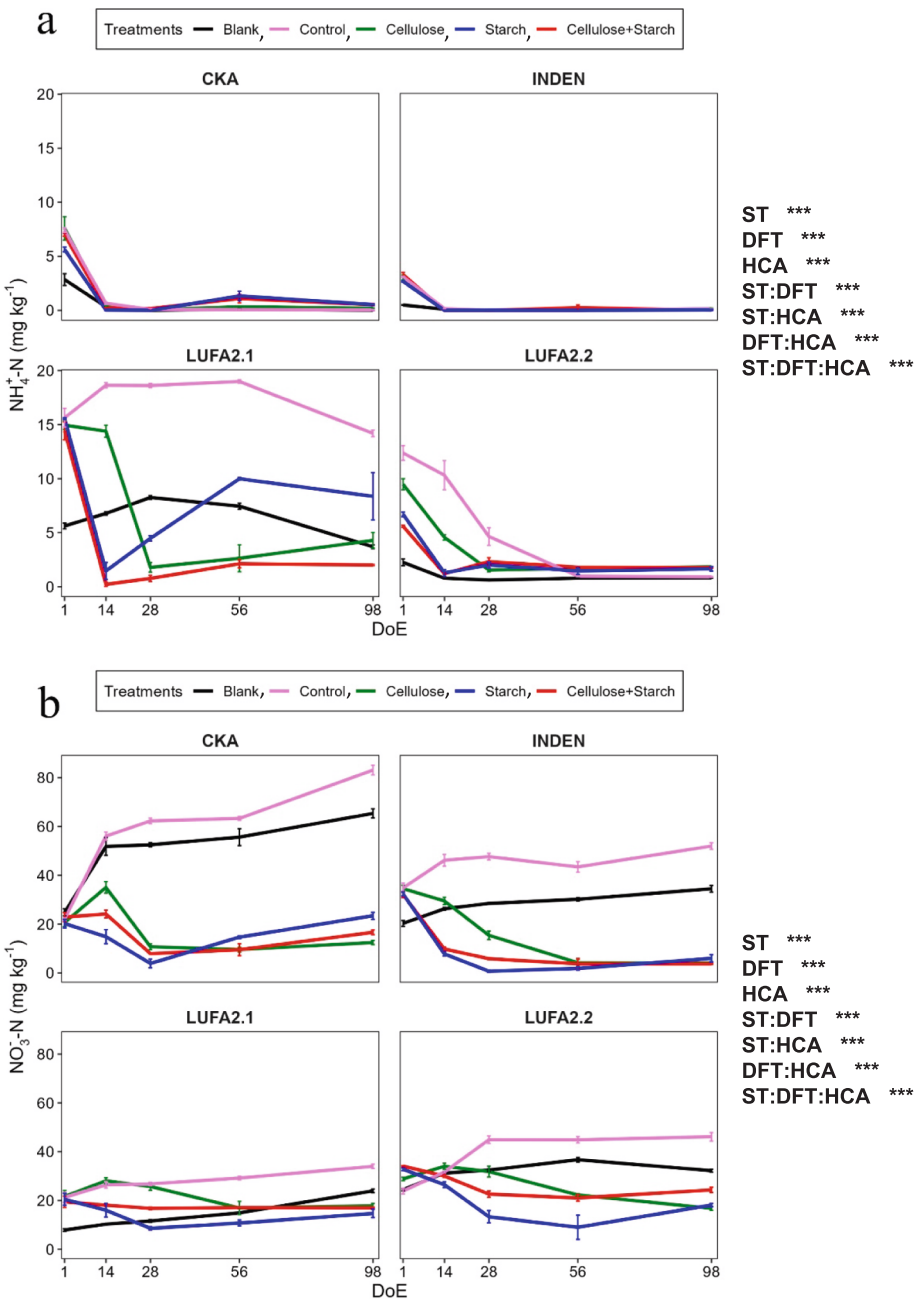


Fig. 1. Dynamics of NH_4^+-N (a), and NO_3^--N (b) in the four soils experiment, differentiated by treatments. Data points are mean values of treatment replicates (n = 4). Standard deviations of mean values (n = 4) are displayed. Significant treatment and interaction effects as revealed by repeated measured ANOVA from Table S1 are given on the right, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

soils. The cumulative N_2O emissions resembled only a small fraction of the N_{min} content and had no significant impact on the soil N pool. The highest cumulative N_2O emission was observed in the Cellulose treatment of the LUFA 2.1 soil, reaching 164.2 mg N m^{-2} (equivalent to 1.642 kg N ha^{-1}, i.e., 3 % of applied N) (Fig. S4, $p < 0.05$). In comparison, N_2O emissions of the other treatments ranged from 1.94 to 18.61 mg N m^{-2} (equivalent to 0.019–0.186 kg N ha^{-1}, i.e., 0.03 %-0.33 % of applied N) (Fig. S4).

3.4. HCA-mediated post-harvest N transfer between soil N pools

In general, the distribution of ^{15}N in the various soil N pools was found to be significantly affected by the interactions between soils and addition of the HCAs (Table S1). On DoE 98, the ^{15}N recovery in N_{min} of the $HCA_{N\text{-free}}$ treatments was 25–30 % lower than that of the Control (Table S3; Fig. 4, $p < 0.05$). In addition, the $HCA_{N\text{-free}}$ treatments showed a significantly higher ^{15}N recovery in the N_{ret} fractions compared to the Control in all soils (Fig. 4, $p < 0.05$). However, no significant difference in microbial biomass between HCA-treated and the control soils was observed. Throughout the incubation period, the Cellulose + Starch treatments had the highest average ^{15}N recovery in the N_{ret} pool of the four soils, at 64 %, compared to 48 % for the separate Cellulose and Starch treatments. Furthermore, at the end of the experiment, the ^{15}N recovery in N_{ret} in the Control ranged from 4.4 % to 33.1 %. Meanwhile, the average ^{15}N recovery in N_{ret} in the HCA treatments was 53.9 %, with value of 66 % for CKA, 63 % for INDEN, 46 % for LUFA 2.1 and 39 % for LUFA 2.2, respectively, across the four soils (Fig. 4). Additionally, in CKA and INDEN soils, the N_{min} content in the CEL and C + S treatment

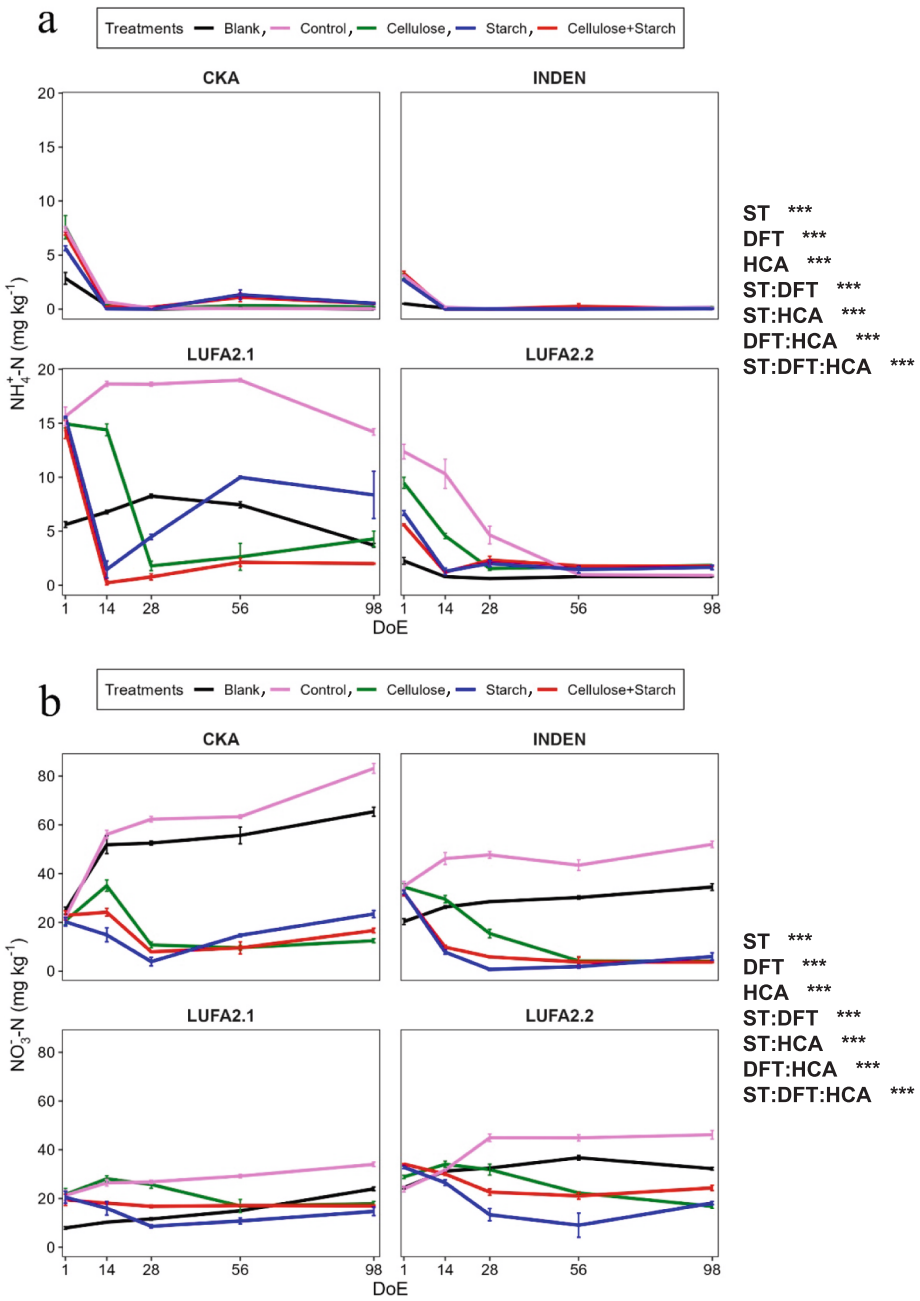


Fig. 2. Dynamics of microbial biomass carbon (C_{mic}) (a) and microbial biomass nitrogen (N_{mic}) (b). Data points are mean values of treatment replicates (n = 4). Standard deviations of mean values (n = 4) are displayed. Significant treatment and interaction effects as revealed by repeated measured ANOVA from Table S1 are given on the right, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

showed a significant negative correlation with the N_{ret} content, but this was not observed in LUFA 2.1 and LUFA 2.2 soils (Fig. S4, a–d, $p < 0.05$). In contrast, the Blank and Control did not show correlations between N_{min} and N_{ret} contents (Fig. S5 a-d, $p > 0.05$). A significant negative correlation between ^{15}N recovery in N_{min} and N_{ret} was found in starch-treated CKA, INDEN, and LUFA 2.1 soils, as well as in cellulose-treated INDEN and LUFA 2.1 soil, but not in LUFA 2.2 soil (Fig. S5, e-h, $p < 0.05$).

The SMLR indicated that SOC was a significant contributor to N_{min} content and ^{15}N recovery in N_{ret} (Table.S6). The results of SEM showed that in CKA and INDEN soils, N_{mic} content exhibits negative feedback on N_{min} content, and indirectly influenced the N_{ret} content and ^{15}N recovery in N_{ret} (Fig. 5a, b, $p < 0.05$). In contrast, in the LUFA soils, N_{mic} and CO_2 emissions had no significant effects on the soil N pools (Fig. 5c, d). In addition, 57 % and 31 % of the variation in N_{min} and ^{15}N recovery in N_{ret} could be explained by soil and microbial variables in SEM, respectively, when the data of the four soils were combined (Fig. 5e). In particular, the $HCA_{N\text{-}free}$ and soil properties were found to significantly influence CO_2 emissions and N_{mic} (Table S1, $p < 0.05$), which in turn exerted an indirect effect on N_{min} and ^{15}N recovery in N_{ret} (Fig. 5e). The standardized total effects indicate that, in addition to HCA, SOC was the most influential factor on N_{min}, with a value of 0.17 (Fig. 5f). In contrast, pH had a minimal effect of −0.02. Conversely, pH exhibited a higher positive correlation with ^{15}N recovery in N_{ret}, with an influence factor of 0.22 (Fig. 5f).

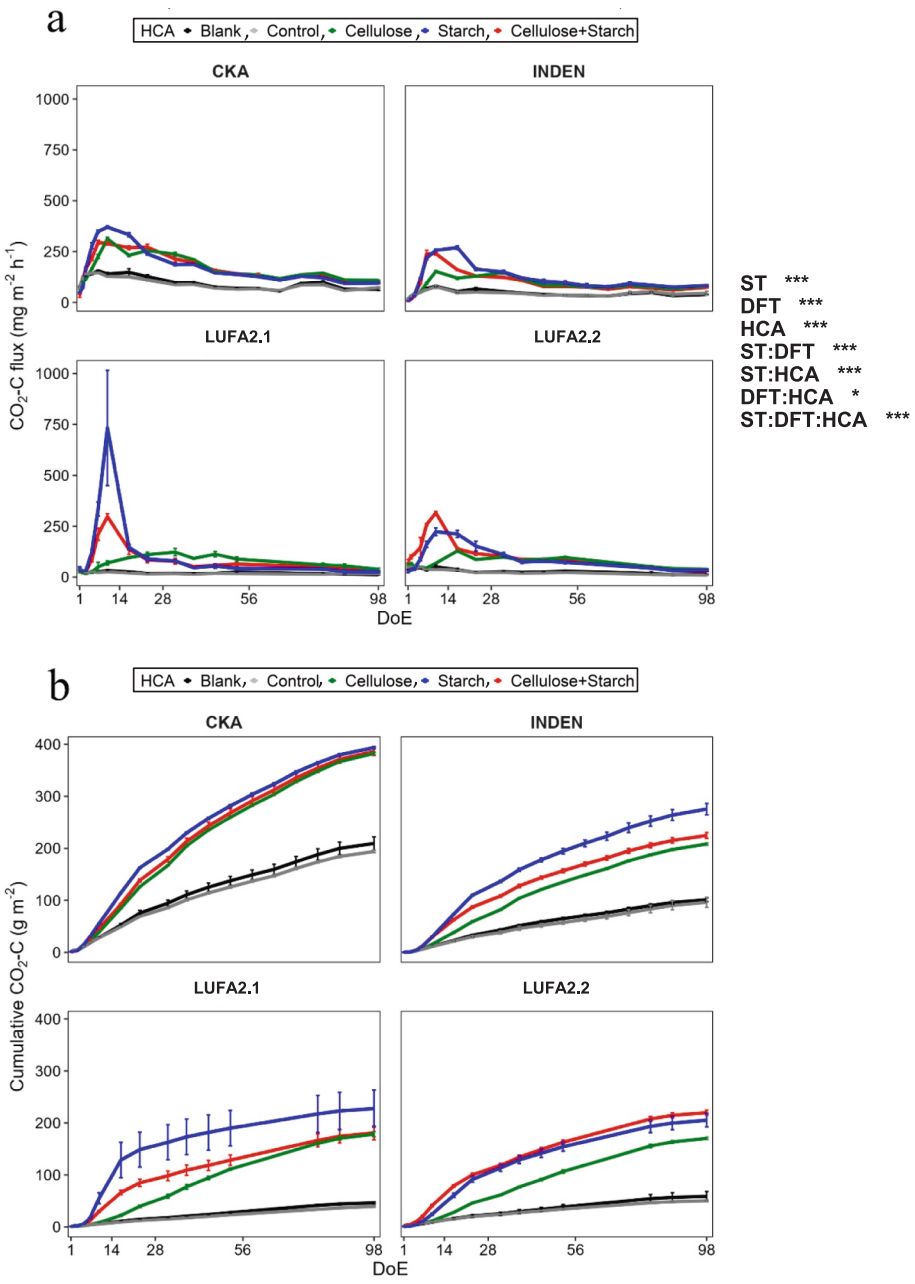


Fig. 3. Dynamics of CO_2 fluxes (a) and the cumulative CO_2 emission (b) in four soil types of the treatments. Data points are mean values of treatment replicates (n = 4). Standard deviations of mean values (n = 4) are displayed. Significant treatment and interaction effects as revealed by repeated measured ANOVA from Table S1 are given on the right, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$.

3.5. Contribution of amino acid compounds to the HCA-driven N immobilization process

The results of the amino acid analysis of the Control, Cellulose and Starch treatments indicated that the AAN accounted for 20–29 % of the N_{ret} at the end of the incubation (Fig. S7a–d). The $HCA_{N\text{-}free}$ treatments exhibited a higher AAN content than the Control in the CKA and LUFA 2.1 soil (Fig. S7 a, c). However, the ^{15}N recovery in AAN accounted for a larger proportion of ^{15}N recovery in N_{ret}, i.e., on average 69 % in CKA and between 31 % and 45 % in the other three soils (Fig. 6). In addition, the ^{15}N recovery of the AAN pool was higher than in the N_{min} pool in the $HCA_{N\text{-}free}$ treatments. The addition of $HCA_{N\text{-}free}$ increased the ^{15}N recovery of AAN significantly ($p < 0.05$), except in CKA. Furthermore, the ^{15}N recovery in AAN in the CKA soil was higher than in the other soils, reaching 24 %, 41 % and 36 % in the Control, Cellulose and Starch treatments, respectively. The second highest recovery of ^{15}N was observed in LUFA 2.2, reaching 8 %, 27 % and 15 % in Control, Cellulose and Starch, respectively. Compared to the Starch treatment, Cellulose resulted in a significantly higher ^{15}N recovery of the AAN pool in LUFA 2.2 ($p < 0.05$), while this recovery was only marginally higher in other three soils ($p > 0.05$). Furthermore, the ^{15}N recovery per unit N was higher in the AAN pool than in N_{ret}, and this difference was even more pronounced in $HCA_{N\text{-}free}$ treatments (Fig. S7e–h).

4. Discussion

4.1. Effect of the N-free HCAs on N_{min} immobilization in contrasting soils

The incorporation of $HCA_{N\text{-}free}$ resulted in a pronounced decline in N_{min} and an increase in CO_2 emissions in comparison to the Control

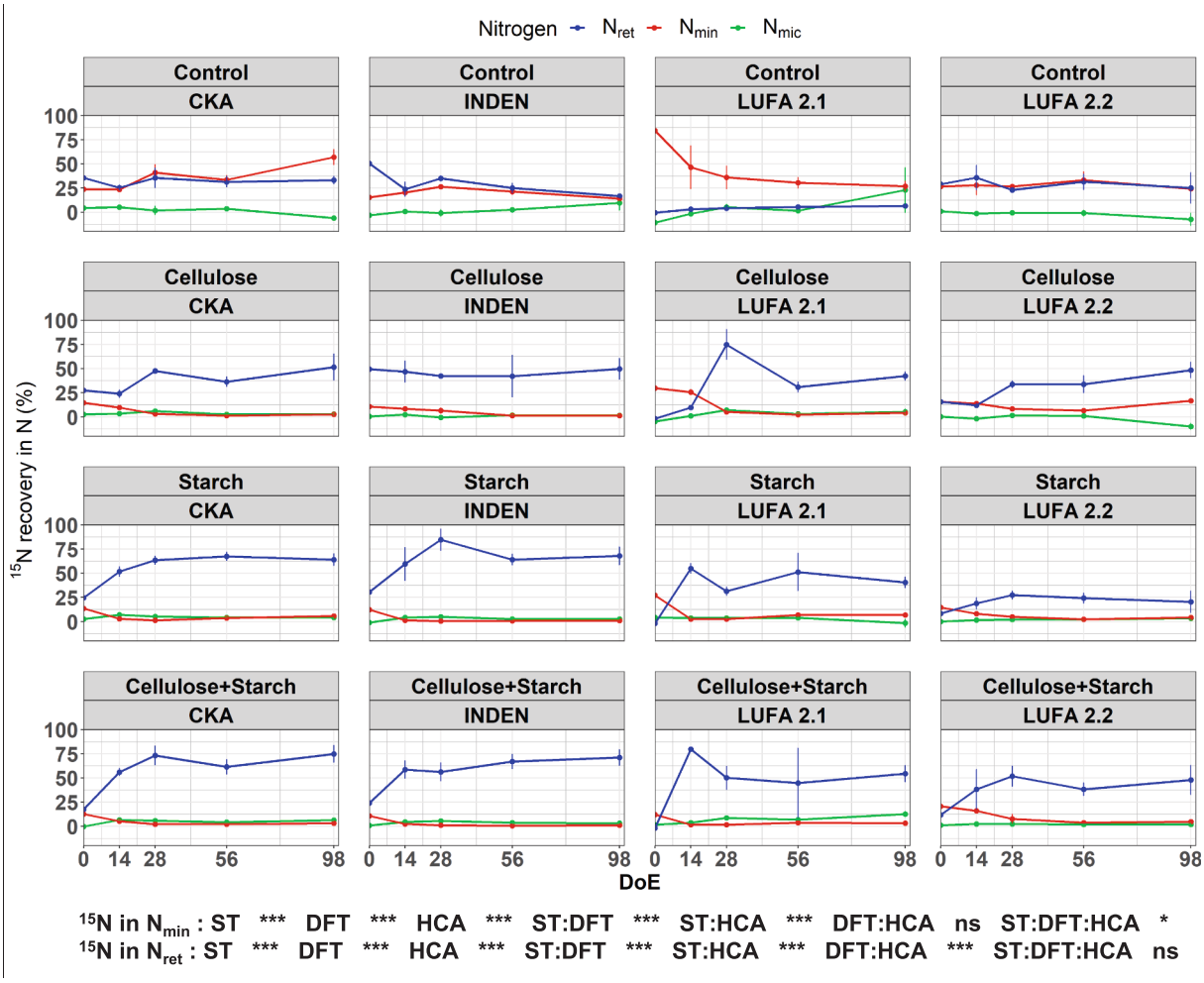


Fig. 4. ^{15}N recovery rates in N_{ret} (blue), N_{min} (red) and N_{mic} (green) in treatments. N_{ret}: retained organic N; N_{min}: mineral N; N_{mic}: microbial biomass N; DoE: Days of experiment. Data are mean values (n = 4), and error bars display standard deviation. Significant treatment and interaction effects as revealed by repeated measured ANOVA from Table S1 are given on the bottom, with *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Figs. 1 and 3), indicating that the microbial communities in the experimental soils were constrained by C availability (Bonner et al., 2018; Siegwart et al., 2023). The application of $HCA_{N\text{-}free}$ has been found to result in two distinct mechanisms that contribute to the observed decrease in N_{min}. First, the presence of C from $HCA_{N\text{-}free}$ shifts microbial mineralization of native SOM to $HCA_{N\text{-}free}$. Second, the availability of C from the $HCA_{N\text{-}free}$ satisfies the microbial C demand, allowing microbes to increase the utilization of available N_{min} to overcome N limitation. Consequently, sufficient C facilitates microbial N assimilation and regulates the mineralization of native SOM (Gunina & Kuzyakov, 2015). The substantial contribution of CO_2 and N_{mic} to N_{min} in the SEM underscores the enhanced assimilation of N_{min} by the microorganisms (Fig. 5e), indicating that the mineralization by soil microbes was preferentially directed towards available HCA, a finding that is consistent with previous studies (Kuzyakov et al., 2000; Wei et al., 2020).

Given that the cumulative emissions of N_2O-N were generally only 0.33 % of the applied N, and that N_{mic} was negatively correlated with the N_{min} in HCA treatments (Fig. S6b), the results verified that the remaining N_{min} had been stabilized in the soil by microbial activity (Fig. 2 and S4). However, the N_{min} results varied depending on the soils and the specific $HCA_{N\text{-}free}$ used. The greatest decrease in N_{min} content of the $HCA_{N\text{-}free}$ treatments was observed in the CKA and INDEN soils, which had higher pH values than the other two soils (Fig. 1). Specifically, the addition of starch to these soils resulted in a larger increase in CO_2 emission and C_{mic} than after addition of cellulose, which correlated with the accelerated decline in N_{min} during DoE 0–28. It can be attributed to the fact that starch is more accessible to microbes than cellulose, and our result is consistent with previous studies (German et al., 2011; Mizuta et al., 2015). Moreover, the Starch treatment sustained the N_{min} retention only to DoE 28 or DoE 56, after which N_{min} increased again until the end of the incubation period (Fig. 1). The concurrent decrease in C_{mic} accompanied by the increase in N_{min} can be attributed to the depletion of starch, with microbial processes shifting back from N_{min} assimilation to mineralization of dead microbial biomass and SOM, thereby producing instead of consuming N_{min} (Fig. 2), in line with previous reports (Allison et al., 2014). While microbial community data were not included in the present study, previous studies have shown that bacteria play the key role in the decomposition of starch (Kamble & Bååth, 2016), and the turnover product is considered to be more easily mineralized due to the lower C/N ratio. In contrast, N_{min} decreased more slowly in the Cellulose treatments of the four soils than in the Starch and Cellulose + Starch mixture treatments between DoE 0 to 28 (Fig. 1). This can be attributed to the activity of fungi, which have been described to have a strong capability of nutrient accumulation under conditions of nutrient deficiency, but also at lower rates than bacteria (Kang et al., 2024; Zheng et al., 2023). Previous studies have reported that complete hydrolysis of cellulose into glucose units is mainly facilitated by fungi and requires more time to activate the required enzymes (Baldrian et al.,

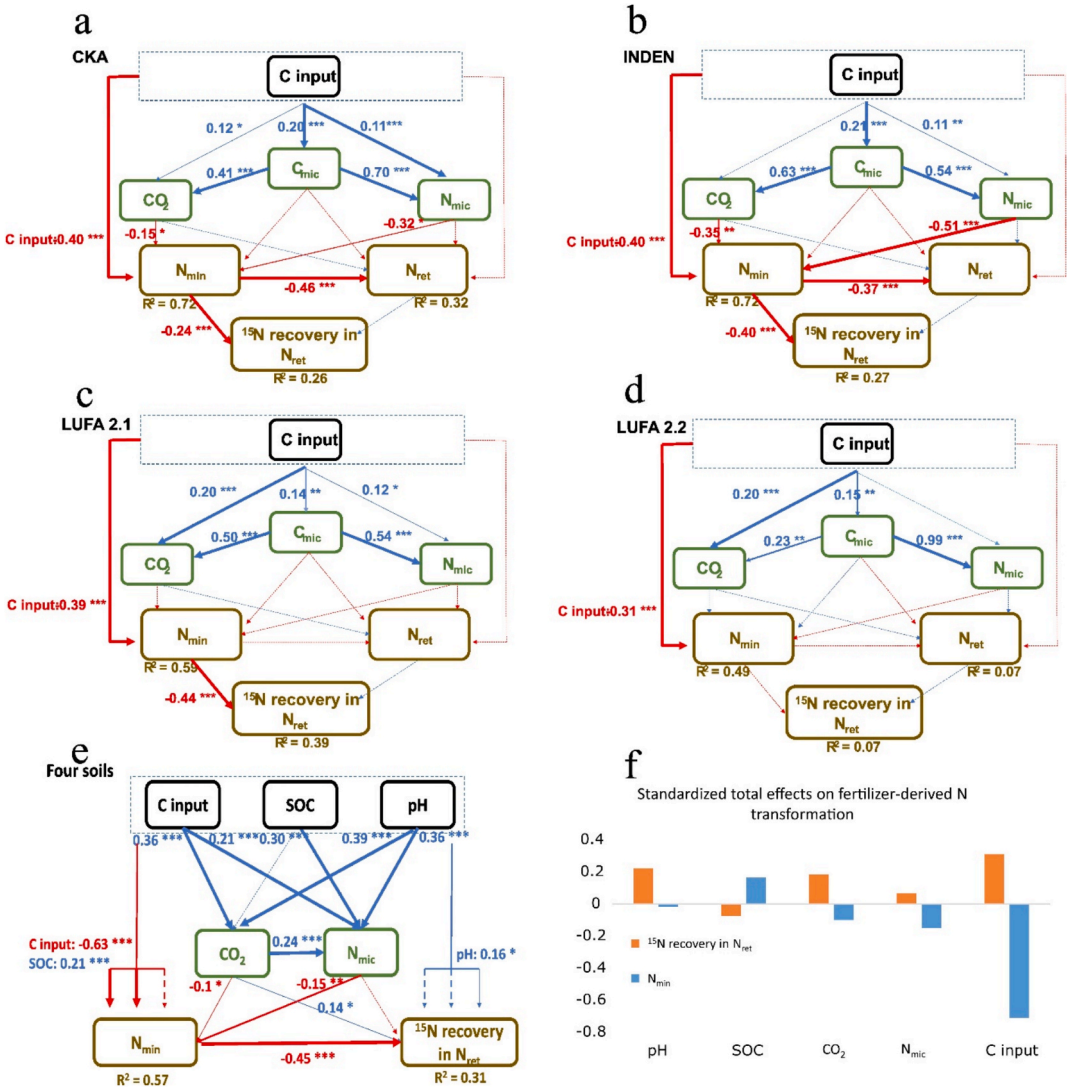


Fig. 5. Structural equation model explaining the multivariate effects on N_{min} and ^{15}N transformation in N_{ret} in CKA soil (a), INDEN soil (b), LUFA 2.1 soil (c), LUFA 2.2 soil (d) and the entire soil dataset (e). The total effects of controlling variables in four soils is shown in (f). The numbers adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship. Red lines represent a negative correlation, while blue lines represent a positive correlation. Arrow width is proportional to the magnitude of the path coefficients; the solid and dashed arrows indicate significant and non-significant relationships, respectively. The proportion of variance explained (R^2) appears next to each response variable. SOC: soil organic C; C_{mic}: microbial biomass C; N_{mic}: microbial biomass N; N_{min}: mineral N; N_{ret}: retained organic N. Significance levels are as follows: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. Goodness-of-fit statistics are shown below the modeling frames. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2012; Vidal et al., 2021).

In addition, we observed that pH exerted a regulatory effect on N_{min}, possibly by influencing microbial activity (Fig. 5f). A substantial increase in starch degradation was observed in the acidic (LUFA) soils, as evidenced by increased CO_2 flux and DOC release. Conversely, the results of the SEM analysis revealed that the relationship of N_{mic} and CO_2 to N_{min} was negative in the CKA and INDEN soils but absent in the LUFA 2.1 and LUFA 2.2 soils (Fig. 5a–d). The findings suggest that microbial N assimilation had a relatively minor effect on N_{min} in acidic soils compared to neutral pH soils due to the suppression of microbial assimilation and growth (Malik et al., 2018; Ontman et al., 2023). The fact that the decline in N_{min} was more pronounced in the INDEN soil compared to the CKA soil, while no such decline occurred in the acidic soils, can be attributed to SOC, which showed a positive effect on N_{min} in the SEM results (Fig. 5f). In general, SOC reflects the native SOM content and the rate of C saturation. High SOC led to greater N mineralization of SOM, which in turn led to a more pronounced increase in N_{min} and decrease in ^{15}N atom% in N_{min} in soils without $HCA_{N\text{-free}}$ (Table S7). Conversely, the microbial C demand in soil with low SOC content is preferentially met by the supply of HCA, which is why the N_{min} in the INDEN soil was the lowest among all soils and with the smallest changes in ^{15}N atom% of N_{min} (Table S7). Since the mineralization of SOM depends on microbial activity, the influence of SOC on the reduction of N_{min} was lower in acidic soils. In short, the addition of $HCA_{N\text{-free}}$ to post-harvest soils has been shown to result in a decrease in N_{min} without the emission of considerable amounts of N gases (Fig. S4). Additionally, the application of starch to post-harvest soils resulted in a more rapid decline in N_{min} than after the addition of cellulose. In contrast, cellulose addition resulted in a longer-lasting reduction in N_{min} levels compared to starch over a period of 98 days.

4.2. Effect of N-free HCAs on microbial-mediated organic N retention in contrasting soils

Our results demonstrate that the conversion of ^{15}N-labelled N_{min} to N_{ret} was the primary driver of N_{min} retention, rather than storage in N_{mic}

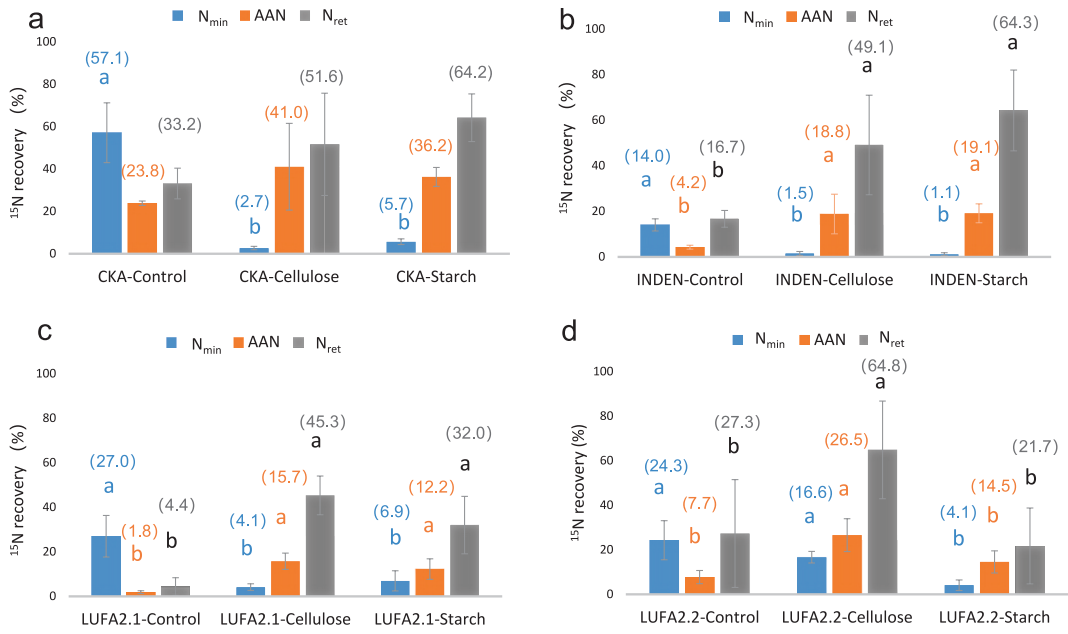


Fig. 6. ^{15}N recovery rates in N_{min} (blue), AAN (orange) and N_{ret} (grey) in Control, Cellulose and Starch treatments in DoE 98. Four figures represented CKA (a), INDEN (b), LUFA2.1 (c), LUFA2.2 (d) soils, respectively. N_{min}: mineral N; AAN: amino acid N; N_{ret}: retained organic N. The Letters of three colours indicate the significant differences of the three treatments in each N pool ($p < 0.05$), and no letter indicates no significant difference between the three treatments. Data in parentheses are average value ($n = 4$). Standard deviations of mean values ($n = 4$) are displayed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4). Compared with the low ^{15}N recovery rates in the Control (range: 4.4 % to 33.1 %), the $HCA_{N\text{-}free}$ treatments showed higher ^{15}N recovery rates at the end of the experiment, ranging from 39 % to 66 % (Fig. 4). It has been demonstrated that the addition of exogenous C can facilitate the increase in soil N_{ret} within a few days, and our findings have confirmed that a large proportion of N_{min} derived from excess fertilizer and SOM mineralization can be immobilized in post-harvest agricultural soils with different properties, but that this process can last longer than previously observed (Chen et al., 2024a, b).

Our ^{15}N recovery results indicate that after 96 days of experiment, 39–66 % of the fertilizer-derived N was immobilized in the stable N_{ret} pool of the soils supplemented with $HCA_{N\text{-}free}$ (Fig. 4, Table S5). Emission of CO_2 indicated microbial respiration associated with the recovery of ^{15}N in N_{ret} (Fig. 5f). One potential explanation is that microbial turnover was accelerated by the additional C provided, resulting in the production of greater quantities of N-rich microbial necromass (Buckeridge et al., 2020). Specifically, the results in Fig. 6 showed that a greater proportion of the fertilizer-derived N was converted to N_{ret} in the $HCA_{N\text{-}free}$-amended soils, particularly in the CKA and INDEN soils (Fig. 6 a, b), compared to the LUFA 2.1 and 2.2 soils (Fig. 6 c, d). The SEM results suggest that pH had the greatest influence on ^{15}N recovery in N_{ret} (Fig. 5f). The effect of starch on N_{ret} immobilization was less pronounced than that of cellulose in the LUFA soils (Fig. 4). Our results also suggest that the transformation of N_{min} to N_{ret} was dominated by cellulose in the Cellulose + Starch treatment of the LUFA soils, resulting from the greater stabilization of fungal necromass in acidic environments (Xue et al., 2023). This finding is consistent with the known positive response of fungi to acidic conditions, as reported in other studies (Donnelly et al., 1990; Teng et al., 2023; Ye et al., 2022).

Moreover, a significant negative correlation was found between ^{15}N recovery in N_{min} and in N_{ret} in the $HCA_{N\text{-}free}$ treatments in the CKA, INDEN and LUFA 2.1 soils (Fig. S5e–j). However, this phenomenon was not observed in the LUFA 2.2 soil, indicating that the LUFA 2.2 soil had a reduced capacity to immobilize newly formed N_{ret} compared to the other three soils. It can be assumed that the elevated SOC levels of LUFA 2.2 (1.72 % SOC), may have resulted in a reduction of adsorption sites for N_{ret} on the soil mineral surfaces, given the fact that this soil had 84 % to 390 % higher SOC than the other soils (Knicker, 2004; Mizuta et al., 2015; Spohn, 2024). Furthermore, the comparatively high C_{mic} and reduced CO_2 emission in the LUFA 2.2 soil indicated a higher CUE, potentially the result of a reduced production of extracellular organic substances (Tao et al., 2023). Our findings, supported by other studies, suggest that soils with low SOC may be more effective at stabilizing newly formed N-rich compounds and protecting them from mineralization than soils already rich in SOC (Bonanomi et al., 2019; Castellano et al., 2012; Stewart et al., 2008). Our results also demonstrate that approximately 50 % of fertilizer-derived N can be immobilized as N_{ret} in the post-harvest period with microbial mediation, for a duration of more than three months. However, certain soil properties, such as soil pH and high SOC, may influence the conversion of specific $HCA_{N\text{-}free}$ substances, potentially impeding the N stabilization process.

4.3. *The role of amino acid in $HCA_{N\text{-}free}$ regulated N immobilization*

Consistent with previous studies, our results indicate that amino acids are an important component of the soil stable N_{ret} pool, accounting for up to 30 % (Fig. S7a–d) (Li et al., 2023; Senwo & Tabatabai, 1998). Given that free amino acids in soil solution usually constitute a minor proportion (less than 1 %) of the total soil N, the extracted amino acids in this study are considered to be predominantly in mineral-associated form, or in the form of hardly soluble polypeptides or proteins (Rothstein, 2010; Warren, 2014). The AAN is therefore considered to be part of N_{ret} pool. Our study demonstrates that the incorporation of $HCA_{N\text{-}free}$ resulted in an increase of amino acid content following the 98-day post-harvest interval (Fig. 6). This phenomenon can be attributed to the promotion of microbial turnover facilitated by the exogenous C. In the presence of sufficient C in the soil, microbes typically utilize N_{min} or small N-containing organic compounds, such as amino acids, as N sources. Subsequently, N is returned to the soil N pool as small-molecule N compounds or necromass fragments (Geisseler et al., 2010). However, the difference in amino acid content between the Control and $HCA_{N\text{-}free}$ treatments was less pronounced than in previous field studies (Fig. S7a–d), which underlines that the return of crop residues can substantially increase amino acid levels throughout the entire growing

season (Wang et al., 2022; Xu et al., 2024). This difference can be attributed to the shorter period and the absence of crops in our post-harvest study.

We also observed that the ^{15}N recovery of AAN was significantly higher in $HCA_{N\text{-free}}$ treatments than in the Control (Fig. 6). This result is consistent with the result of ^{15}N in N_{ret}, indicating that the addition of $HCA_{N\text{-free}}$ alleviates soil C deficiency, enabling microorganisms to assimilate excess N_{min} into amino acid compounds and store them in the N_{ret} pool. The results demonstrate that the newly synthesized AAN in $HCA_{N\text{-free}}$ treatments dominated the N_{ret} pool, accounting for 30 % to 90 % of N_{ret}, which is higher than the 15–30 % reported in a previous study (Lu et al., 2018). The high contribution of new synthesized AAN to N_{ret} can be explained by the fact that amino acids are important structural components of N-rich compounds, including the cell walls, which accumulate in soil as microbial necromass (Vollmer et al., 2008; Zhang and Amelung, 1996).

With the exception of the LUFA 2.2 soil, no significant differences in ^{15}N recovery in AAN were observed between the Cellulose and Starch treatments. This suggests that the different microbial availability of cellulose and starch did not substantially influence the substantial role of new synthesized AAN in the N immobilization in the N_{ret} pool. It was reported previously that glucose addition can significantly increase amino acid content in the soil (Zhang et al., 2015), indicating that the accumulation of new synthesized amino acid compounds is affected by addition of HCA.

The CKA and LUFA 2.2 soils, which had higher SOC content than the INDEN and LUFA2.1 soils, were characterized by a greater conversion of N_{min} to amino acid compounds (Fig. S7e–h). This greater conversion can be attributed to the promotion of microbial community assimilation capacity in soils with high carbon content, as evidenced by the C_{mic} data (Fig. 2). In contrast, in the INDEN and LUFA 2.1 soils with low SOC content, we observed that the addition of $HCA_{N\text{-free}}$ prompted microorganisms to invest more of newly assimilated N_{min} in amino acid compounds, resulting in a higher proportion of newly synthesized AAN in the amino acid pool (Fig. S7e–h). This can be explained by the fact that microbial-derived amino acids preferentially attach to mineral surfaces rather than to areas involving organo-mineral interactions (Kopittke et al., 2018). In comparison to CKA and INDEN, the low-pH sandy soils LUFA 2.1 and LUFA 2.2 were observed to exhibit reduced ^{15}N recovery in AAN pool, suggesting that pH and soil texture may also influence the N pool of newly synthesized amino acids. Furthermore, the AAN results we obtained at DoE 98 illustrate that amino acids produced after HCA addition can stabilized in SOM, rather than in the living microbes, as shown by higher N_{ret} than N_{mic} (Fig. 4). This finding is in line with a previous study in which peptides contributed to the formation of SOM over a 224-day incubation period (Miltner et al., 2009). It has been reported that amino acids and peptides decompose more slowly than other N-free organic compounds, resulting in stabilization in the soil matrix (Spohn, 2024).

5. Conclusion

This study shows that N-free, labile organic C substances, such as cellulose and starch, can significantly improve post-harvest N retention in soils. After 98 days, up to 64 % of residual N_{min} remained in the soil's stable N_{ret} pool, with 12.2–41.0 % of the immobilized N_{min} remaining in the soil as microbial-derived amino acid compounds. Starch resulted in a more rapid decline in N_{min}, while cellulose maintained lower N_{min} for a longer period of time. Soil properties, particularly pH and SOC content, strongly influenced immobilization efficiency and N_{min} stability. These results highlight the practical potential of labile organic C amendments for long-term N conservation, although field trials are needed to validate this effect under real agronomic conditions.

CRediT authorship contribution statement

Kerui Zhao: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Rüdiger Reichel:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Holger Wissel:** Methodology. **Xiao Lu:** Writing – review & editing. **Nicolas Brüggemann:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Funding

This study was supported by the BMBF in the frame of the BonaRes project INPLAMINT (FKZ 031B1062A). Kerui Zhao acknowledges his funding by the Chinese Scholarship Council (scholarship no. 202008410232).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

All the authors would like to thank Antonio Voss, Daniela Gesekus, Lutz Weihermüller and Franz Leistner for their kind support during the microbial biomass, mineral N analysis, soil texture, and gas emission analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117500.

Data availability

The raw data supporting the conclusions of the article will be made available by the authors, without undue restriction, to peers upon request.

References

Allison, S.D., Chacon, S.S., German, D.P., 2014. Substrate concentration constraints on microbial decomposition. Soil Biol. Biochem. 79, 43–49. https://doi.org/10.1016/j.soilbio.2014.08.021.

Baldrian, P., Kolaiřík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 6 (2), 248–258. https://doi.org/10.1038/ismej.2011.95.

Beeckman, F., Motte, H., Beeckman, T., 2018. Nitrification in agricultural soils: impact, actors and mitigation. Curr. Opin. Biotechnol. 50, 166–173.

Biernat, L., Taube, F., Vogeler, I., Reinsch, T., Kluß, C., Loges, R., 2020. Is organic agriculture in line with the EU-Nitrate directive? On-farm nitrate leaching from organic and conventional arable crop rotations. Agric. Ecosyst. Environ. 298. https://doi.org/10.1016/j.agee.2020.106964.

Blattmann, T.M., Ishikawa, N.F., 2020. Theoretical amino acid-specific radiocarbon content in the environment: hypotheses to be tested and opportunities to be taken. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.00302.

Blattmann, T.M., Montluçon, D.B., Haghipour, N., Ishikawa, N.F., Eglinton, T.I., 2020. Liquid chromatographic isolation of individual amino acids extracted from sediments for radiocarbon analysis. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.00174.

BMEL, & BMU. (2020). Nitratbericht 2020. Gemeinsamer Bericht der Bundesministerien für Umwelt, Naturschutz und nukleare Sicherheit (BMU) sowie für Ernährung und Landwirtschaft (BMEL).

Bonanomi, G., Sarker, T.C., Zotti, M., Cesarano, G., Allevato, E., Mazzoleni, S., 2019. Predicting nitrogen mineralization from organic amendments: beyond C/N ratio by 13C-CPMAS NMR approach. Plant Soil 441 (1–2), 129–146. https://doi.org/10.1007/s11104-019-04099-6.

Bonner, M.T.L., Shoo, L.P., Brackin, R., Schmidt, S., 2018. Relationship between microbial composition and substrate use efficiency in a tropical soil. Geoderma 315, 96–103.
Buckeridge, K.M., La Rosa, A.F., Mason, K.E., Whitaker, J., McNamara, N.P., Grant, H.K., Ostle, N.J., 2020. Sticky dead microbes: rapid abiotic retention of microbial necromass in soil. Soil Biol. Biochem. 149. https://doi.org/10.1016/j.soilbio.2020.107929.
Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biol. Biochem. 35 (1), 29–36. https://doi.org/10.1016/S0038-0717(02)00233-X.
Castellano, M.J., Kaye, J.P., Lin, H., Schmidt, J.P., 2012. Linking carbon saturation concepts to nitrogen saturation and retention. Ecosystems 15 (2), 175–187. https://doi.org/10.1007/s10021-011-9501-3.
Chen, Z., Liu, Y., Wu, L., Wang, J., Elrys, A.S., Uwiragiye, Y., Tang, Q., Jing, H., Cai, Z., Müller, C., Cheng, Y., 2024a. Unveiling ammonium concentration ranges that determine competition for mineral nitrogen among soil nitrogen transformations under increased carbon availability. Soil Biol. Biochem. 196. https://doi.org/10.1016/j.soilbio.2024.109495.
Chen, Z., Zhang, H., Tu, X., Wang, J., Elrys, A.S., Tang, Q., Zhang, J., Cai, Z., Cheng, Y., 2024b. C/N ratio of high-organic C materials is a poor predictor of microbial nitrate immobilization potential in a nitrate-rich soil: an 15N incubation study. Soil Tillage Res. 238. https://doi.org/10.1016/j.still.2024.106019.
Cheng, C.-N., 1975. Extracting and desalting amino acids from soils and sediments: evaluation of methods. Soil Biol. Biochem. 7 (4–5), 319–322.
Cheng, C.-N., Shufeldt, R.C., Stevenson, F.J., 1975. Amino acid analysis of soils and sediments: extraction and desalting. Soil Biol. Biochem. 7 (2), 143–151.
Chenu, C., Le Bissonnais, Y., Arrouays, D., 2000. Organic matter influence on clay wettability and soil aggregate stability. Soil Sci. Soc. Am. J. 64 (4), 1479–1486. https://doi.org/10.2136/sssaj2000.6441479x.
Chikowo, R., Mapfumo, P., Nyamugafata, P., Giller, K.E., 2004. Mineral N dynamics, leaching and nitrous oxide losses under maize following two-year improved fallows on a sandy loam soil in Zimbabwe. Plant Soil 259 (1–2), 315–330. https://doi.org/10.1023/B:PLSO.0000020977.28048.fd.
Clocchiatti, A., Hannula, S.E., Hundscheid, M.P.J., Klein Gunnewiek, P.J.A., de Boer, W., 2023. Utilizing woody materials for fungal-based management of soil nitrogen pools. Appl. Soil Ecol. 181. https://doi.org/10.1016/j.apsoil.2022.104663.
Craig, M.E., Mayes, M.A., Sulman, B.N., Walker, A.P., 2021. Biological mechanisms may contribute to soil carbon saturation patterns. Glob. Chang. Biol. 27 (12), 2633–2644. https://doi.org/10.1111/gcb.15584.
Deng, F., Liang, C., 2022. Revisiting the quantitative contribution of microbial necromass to soil carbon pool: stoichiometric control by microbes and soil. Soil Biol. Biochem. 165, 108486.
Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnol. Oceanogr. Methods 6 (6), 230–235. https://doi.org/10.4319/lom.2008.6.230.
Donnelly, P.K., Entry, J.A., Crawford, D.L., Cromack, K., 1990. Cellulose and lignin degradation in forest soils: response to moisture, temperature, and acidity. Microb. Ecol. 20 (1), 289–295. https://doi.org/10.1007/BF02543884.
Elrys, A.S., Chen, Z., Wang, J., Uwiragiye, Y., Helmy, A.M., Desoky, E.-S.-M., Cheng, Y., Zhang, J., Cai, Z., Müller, C., 2022. Global patterns of soil gross immobilization of ammonium and nitrate in terrestrial ecosystems. Glob. Chang. Biol. 28 (14), 4472–4488.
Fanin, N., Alavoine, G., Bertrand, I., 2020. Temporal dynamics of litter quality, soil properties and microbial strategies as main drivers of the priming effect. Geoderma 377. https://doi.org/10.1016/j.geoderma.2020.114576.
Fry, B. (2006). Using Stable Isotope Tracers. In *Stable Isotope Ecology*. https://doi.org/10.1007/0-387-33745-8_3.
Geisseler, D., Horwath, W.R., Joergensen, R.G., Ludwig, B., 2010. Pathways of nitrogen utilization by soil microorganisms - a review. Soil Biol. Biochem. 42 (12), 2058–2067. https://doi.org/10.1016/j.soilbio.2010.08.021.
German, D.P., Chacon, S.S., Allison, S.D., 2011. Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92 (7), 1471–1480. https://doi.org/10.1890/10-2028.1.
Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100.
Henke, J., Böttcher, U., Neukam, D., Sieling, K., Kage, H., 2008. Evaluation of different agronomic strategies to reduce nitrate leaching after winter oilseed rape (*Brassica napus* L.) using a simulation model. Nutr. Cycl. Agroecosyst. 82 (3). https://doi.org/10.1007/s10705-008-9192-0.
Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun. Soil Sci. Plant Anal. 31 (9–10), 1299–1396. https://doi.org/10.1080/00103620009370514.
Hu, Y., Zheng, Q., Noll, L., Zhang, S., Wanek, W., 2020. Direct measurement of the in situ decomposition of microbial-derived soil organic matter. Soil Biol. Biochem. 141. https://doi.org/10.1016/j.soilbio.2019.107660.
Hwang, Y.S., Lenhart, J.J., 2008. Adsorption of C4-dicarboxylic acids at the hematite/water interface. Langmuir 24 (24), 13934–13943.
Islam, M.R., Singh, B., Dijkstra, F.A., 2022. Stabilisation of soil organic matter: interactions between clay and microbes. Biogeochemistry 160 (2), 145–158. https://doi.org/10.1007/s10533-022-00956-2.
Jansson, S.L., Persson, J., 2015. Mineralization and immobilization of soil nitrogen. Nitrogen Agric. Soils 22, 229–252. https://doi.org/10.2134/agronmonogr22.c6.
Jilling, A., Keiluweit, M., Contosta, A.R., Frey, S., Schimel, J., Schnecker, J., Smith, R.G., Tiemann, L., Grandy, A.S., 2018. Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. Biogeochemistry 139 (2), 103–122. https://doi.org/10.1007/s10533-018-0459-5.
Joergensen, R.G., et al., 1995. The fumigation-extraction method to estimate soil microbial biomass: extraction with 0.01 M $CaCl_2$. Agribiol. Res. (Germany) 48 (3).
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEN value. Soil Biol. Biochem. 28 (1), 33–37. https://doi.org/10.1016/0038-0717(95)00101-8.
Johnson, D.W., Cheng, W., Burke, I.C., 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. Soil Sci. Soc. Am. J. 64 (4), 1503–1514.
Kachurina, O.M., Zhang, H., Raun, W.R., Krenzer, E.G., 2000. Simultaneous determination of soil aluminum, ammonium- and nitrate- nitrogen using 1 M potassium chloride. Commun. Soil Sci. Plant Anal. 31 (7–8), 893–903. https://doi.org/10.1080/00103620009370485.
Kamble, P.N., Bååth, E., 2016. Comparison of fungal and bacterial growth after alleviating induced N-limitation in soil. Soil Biol. Biochem. 103, 97–105. https://doi.org/10.1016/j.soilbio.2016.08.015.
Kang, J., Qu, C., Chen, W., Cai, P., Chen, C., Huang, Q., 2024. Organo–organic interactions dominantly drive soil organic carbon accrual. Glob. Chang. Biol. 30 (1). https://doi.org/10.1111/gcb.17147.
Kanter, D.R., Zhang, X., Mauzerall, D.L., 2015. Reducing nitrogen pollution while decreasing farmers' costs and increasing fertilizer industry profits. J. Environ. Qual. 44 (2), 325–335.
Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy 26 (4), 361–375. https://doi.org/10.1016/j.biombioe.2003.08.002.
Knicker, H., 2004. Stabilization of N-compounds in soil and organic-matter-rich sediments—what is the difference? Mar. Chem. 92 (1–4), 167–195.
Koch, H.-J., Stockfisch, N., 2006. Loss of soil organic matter upon ploughing under a loess soil after several years of conservation tillage. Soil Tillage Res. 86 (1), 73–83. https://doi.org/10.1016/j.still.2005.02.029.
Kopittke, P.M., Hernandez-Soriano, M.C., Dalal, R.C., Finn, D., Menzies, N.W., Hoeschen, C., Mueller, C.W., 2018. Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter. Glob. Chang. Biol. 24 (4), 1762–1770. https://doi.org/10.1111/gcb.14009.
Kühling, I., Beiküfner, M., Vergara, M., Trautz, D., 2021. Effects of adapted n-fertilisation strategies on nitrate leaching and yield performance of arable crops in north-western germany. Agronomy 11 (1). https://doi.org/10.3390/agronomy11010064.
Kuzyakov, Y.V., 1997. The role of amino acids and nucleic bases in turnover of nitrogen and carbon in soil humic fractions. Eur. J. Soil Sci. 48 (1), 121–130. https://doi.org/10.1111/j.1365-2389.1997.tb00191.x.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32 (11–12), 1485–1498.
Lavaan, R., 2012. lavaan: an R package for structural equation modeling. J. Stat. Softw. 48 (2), 1–36. https://doi.org/10.18637/jss.v048.i02.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68.
Li, Q., Wang, J., Yuan, H., Chen, B., Zhuang, S., 2023. Climatic effects on soil organic nitrogen fractions and amino acid chirality in paddy soils. Pedosphere 33 (4), 579–588. https://doi.org/10.1016/j.pedsph.2022.06.062.
Li, Y., Harir, M., Lucio, M., Kanawati, B., Smirnov, K., Flerus, R., Koch, B.P., Schmitt-Kopplin, P., Hertkorn, N., 2016. Proposed guidelines for solid phase extraction of Suwannee river dissolved organic matter. Anal. Chem. 88 (13), 6680–6688. https://doi.org/10.1021/acs.analchem.5b04501.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2 (8). https://doi.org/10.1038/nmicrobiol.2017.105.
Liang, Z., Li, W., Yang, S., Du, P., 2010. Extraction and structural characteristics of extracellular polymeric substances (EPS), pellets in autotrophic nitrifying biofilm and activated sludge. Chemosphere 81 (5), 626–632. https://doi.org/10.1016/j.chemosphere.2010.03.043.
Liu, M., Xu, X., Wanek, W., Sun, J., Bardgett, R.D., Tian, Y., Cui, X., Jiang, L., Ma, Z., Kuzyakov, Y., Ouyang, H., Wang, Y., 2024. Nitrogen availability in soil controls uptake of different nitrogen forms by plants. New Phytol. https://doi.org/10.1111/nph.20335.
Lu, C., Chen, H., Teng, Z., Yuan, L., Ma, J., He, H., Chen, X., Zhang, X., Shi, Y., 2018. Effects of N fertilization and maize straw on the dynamics of soil organic N and amino acid N derived from fertilizer N as indicated by 15N labeling. Geoderma 321, 118–126. https://doi.org/10.1016/j.geoderma.2018.02.014.
MacNeill, G.J., Mehrpouyan, S., Minow, M.A.A., Patterson, J.A., Tetlow, I.J., Emes, M.J., 2017. Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. J. Exp. Bot. 68 (16), 4433–4453. https://doi.org/10.1093/jxb/erx291.
Malik, A.A., Puissant, J., Buckeridge, K.M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H.S., Peyton, J.M., Mason, K.E., van Agtmaal, M., Blaud, A., Clark, I.M., Whitaker, J., Pywell, R.F., Ostle, N., Gleixner, G., Griffiths, R.I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. Nat. Commun. 9 (1). https://doi.org/10.1038/s41467-018-05980-1.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196 (1), 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x.
Miltner, A., Kindler, R., Knicker, H., Richnow, H.H., Kästner, M., 2009. Fate of microbial biomass-derived amino acids in soil and their contribution to soil organic matter. Org Geochem. 40 (9), 978–985. https://doi.org/10.1016/j.orggeochem.2009.06.008.
Mizuta, K., Taguchi, S., Sato, S., 2015. Soil aggregate formation and stability induced by starch and cellulose. Soil Biol. Biochem. 87, 90–96.

Montero, G., Coronado, M.A., Garc\'\ia, C., Campbell, H.E., Montes, D.G., Torres, R., Pérez, L., León, J.A., Ayala, J.R., 2018. Wheat straw open burning: Emissions and impact on climate change. Global Wheat Product. https://doi.org/10.5772/intechopen.76031.

Neal, A.L., Barrat, H.A., Bacq-Lebreuil, A., Qin, Y., Zhang, X., Takahashi, T., Rubio, V., Hughes, D., Clark, I.M., Cárdenas, L.M., Gardiner, L.J., Krishna, R., Glendining, M.L., Ritz, K., Mooney, S.J., Crawford, J.W., 2023. Arable soil nitrogen dynamics reflect organic inputs via the extended composite phenotype. Nat. Food 4 (1), 51–60. https://doi.org/10.1038/s43016-022-00671-z.

Ontman, R., Groffman, P.M., Driscoll, C.T., Cheng, Z., 2023. Surprising relationships between soil pH and microbial biomass and activity in a northern hardwood forest. Biogeochemistry 163 (3), 265–277. https://doi.org/10.1007/s10533-023-01031-0.

Pan, W., Tang, S., Zhou, J., Liu, M., Xu, M., Kuzyakov, Y., Ma, Q., Wu, L., 2022. Plant–microbial competition for amino acids depends on soil acidity and the microbial community. Plant Soil 475 (1–2), 457–471. https://doi.org/10.1007/s11104-022-05381-w.

Parikh, S.J., Kubicki, J.D., Jonsson, C.M., Jonsson, C.L., Hazen, R.M., Sverjensky, D.A., Sparks, D.L., 2011. Evaluating glutamate and aspartate binding mechanisms to rutile ($α$-TiO_2) via ATR-FTIR spectroscopy and quantum chemical calculations. Langmuir 27 (5), 1778–1787.

Pihlap, E., Vuko, M., Lucas, M., Steffens, M., Schloter, M., Vetterlein, D., Endenich, M., Kögel-Knabner, I., 2019. Initial soil formation in an agriculturally reclaimed open-cast mining area - the role of management and loess parent material. Soil Tillage Res. 191, 224–237. https://doi.org/10.1016/j.still.2019.03.023.

Power, J.F., Peterson, G.A., 1998. Nitrogen transformations, utilization, and conservation as affected by fallow tillage method. Soil Tillage Res. 49 (1–2), 37–47. https://doi.org/10.1016/S0167-1987(98)00153-6.

Qiu, Y., Zhang, Y., Zhang, K., Xu, X., Zhao, Y., Bai, T., Zhao, Y., Wang, H., Sheng, X., Bloszies, S., Gillespie, C.J., He, T., Wang, Y., Chen, H., Guo, L., Song, H., Ye, C., Wang, Y., Woodley, A., Hu, S., 2024. Intermediate soil acidification induces highest nitrous oxide emissions. Nat. Commun. 15 (1). https://doi.org/10.1038/s41467-024-46931-3.

Rabbi, S.M.F., Warren, C.R., Swarbrick, B., Minasny, B., McBratney, A.B., Young, I.M., 2024. Microbial decomposition of organic matter and wetting–drying promotes aggregation in artificial soil but porosity increases only in wet-dry condition. Geoderma 447. https://doi.org/10.1016/j.geoderma.2024.116924.

Reichel, R., Hänsch, M., Brüggemann, N., 2017. Indication of rapid soil food web recovery by nematode-derived indices in restored agricultural soil after open-cast lignite mining. Soil Biol. Biochem. 115, 261–264. https://doi.org/10.1016/j.soilbio.2017.08.020.

Robertson, G.P., Vitousek, P.M., 2009. Nitrogen in agriculture: balancing the cost of an essential resource. Annu. Rev. Env. Resour. 34 (1), 97–125.

Rothstein, D.E., 2010. Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils. Soil Biol. Biochem. 42 (10), 1743–1750. https://doi.org/10.1016/j.soilbio.2010.06.011.

Said-Pullicino, D., Cucu, M.A., Sodano, M., Birk, J.J., Glaser, B., Celi, L., 2014. Nitrogen immobilization in paddy soils as affected by redox conditions and rice straw incorporation. Geoderma 228–229, 44–53. https://doi.org/10.1016/j.geoderma.2013.06.020.

Saka, S., Ueno, T., 1999. Chemical conversion of various celluloses to glucose and its derivatives in supercritical water. Cellulose 6 (3), 177–191. https://doi.org/10.1023/A:1009232508644.

Scheller, E., Joergensen, R.G., 2008. Decomposition of wheat straw differing in nitrogen content in soils under conventional and organic farming management. J. Plant Nutr. Soil Sci. 171 (6), 886–892. https://doi.org/10.1002/jpln.200700272.

Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R., 2012. Methods in Soil Biology. Springer Science & Business Media.

Senwo, Z.N., Tabatabai, M.A., 1998. Amino acid composition of soil organic matter. Biol. Fertil. Soils 26 (3), 235–242. https://doi.org/10.1007/s003740050373.

Shang, Z., Cui, X., van Groenigen, K.J., Kuhnert, M., Abdalla, M., Luo, J., Zhang, W., Song, Z., Jiang, Y., Smith, P., Zhou, F., 2024. Global cropland nitrous oxide emissions in fallow period are comparable to growing-season emissions. Glob. Chang. Biol. 30 (2). https://doi.org/10.1111/gcb.17165.

Siegwart, L., Piton, G., Jourdan, C., Piel, C., Sauze, J., Sugihara, S., Bertrand, I., 2023. Carbon and nutrient colimitations control the microbial response to fresh organic carbon inputs in soil at different depths. Geoderma 440. https://doi.org/10.1016/j.geoderma.2023.116729.

Sieling, K., Kage, H., 2006. N balance as an indicator of N leaching in an oilseed rape–winter wheat–winter barley rotation. Agric. Ecosyst. Environ. 115 (1–4), 261–269. https://doi.org/10.1016/j.agee.2006.01.011.

Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241, 155–176.

Smith, C.P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., Mccarl, B., Mara, F.O., Rice, C., Scholes, B., Sirotenko, O. (2008). *Chapter 8 Greenhouse Gas Mitigation in Agriculture. March.* https://doi.org/10.1098/rstb.2007.2184.

Spohn, M., 2024. Preferential adsorption of nitrogen- and phosphorus-containing organic compounds to minerals in soils: a review. Soil Biol. Biochem. 194. https://doi.org/10.1016/j.soilbio.2024.109428.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2008. Soil carbon saturation: Evaluation and corroboration by long-term incubations. Soil Biol. Biochem. 40 (7), 1741–1750. https://doi.org/10.1016/j.soilbio.2008.02.014.

Sundermann, G., Wägner, N., Cullmann, A., von Hirschhausen, C.R., Kemfert, C., 2020. Nitrate pollution of groundwater long exceeding trigger value: fertilization practices require more transparency and oversight. DIW Weekly Rep. 10 (8/9), 61–72.

Takano, Y., Kashiyama, Y., Ogawa, N.O., Chikaraishi, Y., Ohkouchi, N., 2010. Isolation and desalting with cation-exchange chromatography for compound-specific nitrogen isotope analysis of amino acids: application to biogeochemical samples. Rapid Commun. Mass Spectrom. 24 (16), 2317–2323. https://doi.org/10.1002/rcm.4651.

Tao, F., Huang, Y., Hungate, B.A., Manzoni, S., Frey, S.D., Schmidt, M.W.I., Reichstein, M., Carvalhais, N., Ciais, P., Jiang, L., Lehmann, J., Wang, Y.P., Houlton, B.Z., Ahrens, B., Mishra, U., Hugelius, G., Hocking, T.D., Lu, X., Shi, Z., Luo, Y., 2023. Microbial carbon use efficiency promotes global soil carbon storage. Nature. https://doi.org/10.1038/s41586-023-06042-3.

Teixeira, E.I., Johnstone, P., Chakwizira, E., de Ruiter, J., Malcolm, B., Shaw, N., Zyskowski, R., Khaembah, E., Sharp, J., Meenken, E., Fraser, P., Thomas, S., Brown, H., Curtin, D., 2016. Sources of variability in the effectiveness of winter cover crops for mitigating N leaching. Agric. Ecosyst. Environ. 220, 226–235. https://doi.org/10.1016/j.agee.2016.01.019.

Teng, Q., Lu, X., Zhang, Q., Cai, L., Sardar, M.F., Li, Y., Abbas, T., Li, Y., Chang, S.X., Li, Y., 2023. Litterfall quality modulates soil ammonium and nitrate supply through altering microbial function in bamboo encroachment of broadleaf forests. Geoderma 437. https://doi.org/10.1016/j.geoderma.2023.116592.

Thapa, R., Mirsky, S.B., Tully, K.L., 2018. Cover crops reduce nitrate leaching in agroecosystems: a global meta-analysis. J. Environ. Qual. 47 (6), 1400–1411. https://doi.org/10.2134/jeq2018.03.0107.

Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M., Lee, Y.K., 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 12 (4), 1072–1083. https://doi.org/10.1038/s41396-018-0082-4.

Vidal, A., Klöffel, T., Guigue, J., Angst, G., Steffens, M., Hoeschen, C., Mueller, C.W., 2021. Visualizing the transfer of organic matter from decaying plant residues to soil mineral surfaces controlled by microorganisms. Soil Biol. Biochem. 160. https://doi.org/10.1016/j.soilbio.2021.108347.

Villa Zabala, C.C., Villa Zabala, C.C., 2020. An overview on starch structure and chemical nature. Starch-Based Nanomater. 3–9.

Vollmer, W., Blanot, D., De Pedro, M.A., 2008. Peptidoglycan structure and architecture. FEMS Microbiol. Rev. 32 (2), 149–167. https://doi.org/10.1111/j.1574-6976.2007.00094.x.

Wang, B., An, S., Liang, C., Liu, Y., Kuzyakov, Y., 2021. Microbial necromass as the source of soil organic carbon in global ecosystems. Soil Biol. Biochem. 162. https://doi.org/10.1016/j.soilbio.2021.108422.

Wang, B.T., Hu, S., Yu, X.Y., Jin, L., Zhu, Y.J., Jin, F.J., 2020. Studies of cellulose and starch utilization and the regulatory mechanisms of related enzymes in Fungi. Polymers 12 (3). https://doi.org/10.3390/polym12030530.

Wang, Z., Liu, Z., Hu, W., Bai, H., Ma, L., Lv, X., Zhou, Z., Meng, Y., 2022. Crop residue return improved soil nitrogen availability by increasing amino acid and mineralization under appropriate N fertilization. Land Degrad. Dev. 33 (13), 2197–2207. https://doi.org/10.1002/ldr.4241.

Warren, C.R., 2014. Response of organic N monomers in a sub-alpine soil to a dry-wet cycle. Soil Biol. Biochem. 77, 233–242. https://doi.org/10.1016/j.soilbio.2014.06.028.

Wei, X., Zhu, Z., Liu, Y., Luo, Y., Deng, Y., Xu, X., Liu, S., Richter, A., Shibistova, O., Guggenberger, G., Wu, J., Ge, T., 2020. C:N:P stoichiometry regulates soil organic carbon mineralization and concomitant shifts in microbial community composition in paddy soil. Biol. Fertil. Soils 56 (8), 1093–1107. https://doi.org/10.1007/s00374-020-01468-7.

Wilhelm, R.C., Lynch, L., Webster, T.M., Schweizer, S., Inagaki, T.M., Tfaily, M.M., Kukkadapu, R., Hoeschen, C., Buckley, D.H., Lehmann, J., 2022. Susceptibility of new soil organic carbon to mineralization during dry-wet cycling in soils from contrasting ends of a precipitation gradient. Soil Biol. Biochem. 169. https://doi.org/10.1016/j.soilbio.2022.108681.

Xing, W., Lu, X., Geng, S., Ding, J., Bai, Y., 2023. Mechanisms underlying the negative effects of nitrogen addition on soil nematode communities in global grassland ecosystems. Geoderma 436. https://doi.org/10.1016/j.geoderma.2023.116564.

Xu, L., Chen, H., Zhou, Y., Zhang, J., Nadeem, M.Y., Miao, C., You, J., Li, W., Jiang, Y., Ding, Y., Li, G., 2024. Long-term straw returning improved soil nitrogen sequestration by accelerating the accumulation of amino acid nitrogen. Agric. Ecosyst. Environ. 362. https://doi.org/10.1016/j.agee.2023.108846.

Xu, X., Shi, Z., Li, D., Rey, A., Ruan, H., Craine, J.M., Liang, J., Zhou, J., Luo, Y., 2016. Soil properties control decomposition of soil organic carbon: results from data-assimilation analysis. Geoderma 262, 235–242. https://doi.org/10.1016/j.geoderma.2015.08.038.

Xue, Z., Qu, T., Li, X., Chen, Q., Zhou, Z., Wang, B., Lv, X., 2023. Different contributing processes in bacterial vs. fungal necromass affect soil carbon fractions during plant residue transformation. Plant Soil 1–19. https://doi.org/10.1007/s11104-023-06277-z.

Ye, G., Fan, J., Hu, H.W., Chen, J., Zhong, X., Chen, J., Wang, D., Wei, X., Lin, Y., 2022. Short-term cellulose addition decreases microbial diversity and network complexity in an Ultisol following 32-year fertilization. Agric. Ecosyst. Environ. 325. https://doi.org/10.1016/j.agee.2021.107744.

Yu, W.H., Li, N., Tong, D.S., Zhou, C.H., Lin, C.X., Xu, C.Y., 2013. Adsorption of proteins and nucleic acids on clay minerals and their interactions: a review. Appl. Clay Sci. 80–81, 443–452. https://doi.org/10.1016/j.clay.2013.06.003.

Zhang, W., Liang, C., Kao-Kniffin, J., He, H., Xie, H., Zhang, H., Zhang, X., 2015a. Differentiating the mineralization dynamics of the originally present and newly synthesized amino acids in soil amended with available carbon and nitrogen substrates. Soil Biol. Biochem. 85, 162–169. https://doi.org/10.1016/j.soilbio.2015.03.004.

Zhang, Xudong, Amelung, W., 1996. Gas chromatograph1c determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biol. Biochem. 28 (9), 1201–1206. https://doi.org/10.1016/0038-0717(96)00117-4.

Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015b. Managing nitrogen for sustainable development. Nature 528 (7580), 51–59. https://doi.org/10.1038/nature15743.

Zhao, K., Reichel, R., Wissel, H., Li, Z., Brüggemann, N., 2025. High-carbon amendments improve post- harvest nitrogen retention in reclaimed soil: results of a laboratory incubation study. Soil Use Manag. https://doi.org/10.1111/sum.70088.

Zheng, T., Miltner, A., Liang, C., Nowak, K.M., Kästner, M., 2023. Turnover of bacterial biomass to soil organic matter via fungal biomass and its metabolic implications. Soil Biol. Biochem. 180. https://doi.org/10.1016/j.soilbio.2023.108995.