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Organic carbon accumulation and microbial activities in arable soils after abandonment: A chronosequence study

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ABSTRACT

Increased plant carbon (C) input into the soils after cropland abandonment results in not only C accumulation, but also higher microbial activities and consequently faster organic matter decomposition. We investigated the link between soil C accumulation and microbial properties in a chronosequence (0–65 years) of post-agricultural self-restoration of Luvisols - the dominating soil type worldwide. Microbial biomass carbon (MBC) and enzyme activities increased in the top soil (0–20 cm) during the 37-year period of self-restoration. Accumulation of microbial biomass was faster than of soil organic C at earlier stages of self-restoration (0–22 years) because of the fast microbial growth induced by labile litter components. The response of microbial activity was more sensitive to land use changes compared to that of soil organic C. Activities of enzymes responsible for C cycle increased more than that of nitrogen (N) and phosphorus (P) cycles at least up to 37 years after abandonment, indicating microbial adaptations to high input of litter with wide C/N and C/P ratios. Similarly, β-xylosidase was the solely enzyme which reached the activity similar to secondary forest soils after 65 years, due to the accumulation of recalcitrant C in litter with cropland abandonment age. The increased C demand was caused by the higher bacterial portion in soil microbial community (based on PLFA composition), which in turn, resulted in a lower microbial biomass C/N ratio. Enzyme stoichiometry revealed that microorganisms were limited by C and N in the topsoil during the self-restoration up to 37 years. Overall, the quantity and quality of plant C inputs, which changed with post-agricultural land restoration, regulated the microbial activity and enzyme production, offering a profound comprehension of ecosystem succession.

1. Introduction

Abandonment of agricultural land is a widespread phenomenon occurring in many regions of the world (Levers et al., 2018; Kurganova et al., 2019; Joshi et al., 2022). Approximately, 2,200,000 km^2 of arable land are now abandoned worldwide, 25% of which are in Russia. Abandoned farmland is experiencing natural succession and often will slowly evolving towards a vegetated condition similar to its pre-agricultural state (Yang et al., 2020), that is self-restoration (Kalinina et al., 2013; Kurganova et al., 2019). As this occurs, it can results in the substantial soil organic carbon (SOC) accumulation (Laganière et al., 2010; Kalinina et al., 2015; Joshi and Garkoti, 2021). For example, in Russia, larger than 90% of abandoned cropland is experiencing self-restoration (Kurganova et al., 2014), and the retardation of cultivation

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caused an enormous SOC accumulation, averaging 548 ± 35 Tg C or 34 Tg yr^{-1} (Schierhorn et al., 2013). However, the effects of SOC accumulation on the recovery of soil functions during self-restoration processes remains unclear.

Microorganisms play a vital role in biogeochemical cycles and are essential to soil functions, especially soil organic matter decomposition and nutrient (i.e., N, P) cycling, and thus provide crucial ecosystem services (Jiao et al., 2018; Kalam et al., 2020; Zhou et al., 2023). Integrating microbial indicators into the study of post-agricultural abandonment could reveal new knowledge regarding the mechanisms that underline microbially driven soil nutrient cycles (Dominguez-Bello et al., 2010; Ovsepyan et al., 2019). Enzymes secreted by microorganisms are also a major indicator reflecting soil functions since microorganisms favor plant growth by driving C and nutrient cycles (Sinsabaugh et al., 2009). It has been documented that the self-restoration of former croplands creates a suitable growth environment for soil microorganisms and further induces an increase in microbial biomass and enzyme activity (Raiesi and Salek-Gilani, 2018; Deng et al., 2018; Jiang et al., 2019; Ovsepyan et al., 2020). For example, the end of cultivation in agroecosystems caused the recovery of soil hydrolase and oxidase activities (i.e., β-glucosidase and phosphatases) in a Mediterranean area (Xiao et al., 2020). However, most studies of soil processes focus on the topsoil layer, where microorganisms are more abundant in response to larger inputs of organic matter from decomposing root litter and rhizodeposition (Peng and Wang, 2016; Gocke et al., 2017). A comprehensive understanding of microbial dynamics within different soil depths have rarely been considered under long-term post-abandonment processes.

Enzymatic stoichiometry can be used to assess microbial resource availability and limitations (i.e., C, N, and P) to microbial and plant growth (Chen et al., 2017; Xiao et al., 2020; Zhou et al., 2022a). The microbial resource limitations can be influenced by agricultural abandonment, owing to the shift in the quality and quantity of plant litter and root exudates caused by the increased plant diversity (Ovsepyan et al., 2020). At early successional chronosequence (3–52 years), soil microorganisms were stronger limited by C and N compared with later stages, whilst it was more seriously limited by P at the late stages (80–120 years) due to the faster loss of P (Jiang et al., 2018). Cui et al. (2019) documented that microorganisms in soils are co-limited by C and P during vegetation restoration in the Loess Plateau of China. Consequently, the alterations of enzymatic stoichiometry because of agricultural abandonment influence the microbial C and nutrients demands. This, in turn, reduces or stimulates microbially-mediated soil biogeochemical processes and ecosystem functions (Jia et al., 2022). Therefore, a more comprehensive quantitative characterization of soil enzyme stoichiometry and resource limitation after the cropland abandonment is urgently required to grasp the microbial resource availability and nutrient limitation.

Bacteria and fungi are important components of soil microbiota, which play a principal role in the maintenance of soil structure, retention of nutrients (Wang et al., 2014; Moreno-Barriga et al., 2017). Bacteria and fungi shift following reforesting processes and are driven by multiple environmental factors, such as SOC content, water, and pH (Spohn et al., 2016; Jiao et al., 2018). Fungi have lower nutrient demands relative to bacteria and show a high C use efficiency in soil with less fertility (Keiblinger et al., 2010). By contrast, bacteria require larger amounts of N and P for maintaining their fast turnover rates (Deng et al., 2018). Hence, the soil nutrient availability can have an impact on microbial composition (Kaiser et al., 2014; Zhang et al., 2016). In post-agricultural lands, soil recovery typically features rapid increases in bacteria count during the first 10–35 years, while fungi may play a predominant role in later stages (Jiang et al., 2019; Li et al., 2020). On the contrary, soil microbial community composition did not change in the Canadian boreal forest (Hahn and Quideau, 2013). These inconsistent results thus call for further studies to enable a deeper understanding of the impact of post-abandonment processes on the soil microbial community composition.

A post-agricultural lands study in European part of Russia was therefore designed to assess how the abandonment of arable soils influences SOC accumulation and subsequent microbial functions. This study aimed to (1) quantify microbial biomass and enzyme activities after cropland abandonment; (2) determine microbial resource limitations in response to cropland abandonment.

2. Materials and methods

2.1. Study site and vegetation during cropland abandonment

The study was conducted in a temperate broad-leaved forest area which was close to the small town of Pushchino (Moscow region, Russia) featuring a temperate continental climate (Kalinina et al., 2018). The mean annual air temperature and precipitation were 5.5–6.0 °C and 565–670 mm in the study area. According to the IUSS Working Group WRB (2014), the soil was classified as Albic Luvisol (Loamic, Cutanic) (Kalinina et al., 2018).

The space-for-time substitution is a commonly applied approach to evaluate long term processes, e.g. recovery of abandoned soils (Walcker et al., 2018). In study site, the chronosequences included one arable plot, and abandoned plot of 9, 22, and 37 years old, as well as one secondary forest of 65 years old which was viewed as a reference plot (Kalinina et al., 2018). The arable plot was not cropped during the sampling year. The abandoned plots of 9 years have belonged to the Experimental Field Station of the Russian Academy of Science (RAS) since 1977. Before abandonment, the sites were used for field experiments. After abandonment, the sites of 9 years were never mowed. The abandoned land of 9 years old was a grassland dominated by *Calamagrostis epigeios*. The abandoned plot of 22 years was part of the collective farm (“kolkhoz”) until 1994. After abandonment, the site was mowed sporadically for at least 10 years. The abandoned plot of 37 years was the part of an RAS. After abandonment in 1980, a field experiment was established by sowing grassland species. Until 2000, young trees were cut manually to keep this site as grassland. The abandoned lands of 22 and 37 years old are a woodland with 25% canopy composed with *Betula* sp., *Populus tremula, Salix* sp. and *Ácer* sp. The forest site has never been previously cultivated, but complete cutting was performed by local people about 65–70 years ago (during the World War II). The secondary forest site was a linden-aspen-maple forest with *Tilia cordata, Populus tremula* and *Acer platanoides* (Kalinina et al., 2018). For the chronosequential approach of this study, sites differing in self-restoration time but comparable in soil texture, climate and land-use history were required. Hence, sampling sites were selected according to information obtained from topographic maps and personal communications with local authorities and indigenous people. In both regions, all fields were located at similar geomorphological positions within a maximal distance of 600 m apart. The differences of the main soil properties (texture, mineralogy, pH, etc.) within each chronosequence were negligible (Table S1).

2.2. Soil sampling

Soils were sampled in June 2016. In each experimental site, four plots with 5 × 5 m were established in the field and separated from each other by buffer stripes of 5–10 m. In each plot, five sites were randomly selected to sample four soil layers including 0–5, 5–10, 10–20, and 20–30 cm by a stainless steel corer (5 cm in diameter). Soils were homogenized and sieved through 2 mm, visible shoot litter and root, as well as stones were picked out. The litter layer was excluded from analysis. Then each sample was separated into two parts. One was stored at 4 °C and was used to measure soil microbial biomass carbon (MBC) and enzyme activity within one weeks. The other part was stored at −20 °C for microbial community analysis. SOC and total N (TN) were determined and results were presented early (Kalinina et al., 2018). Soil

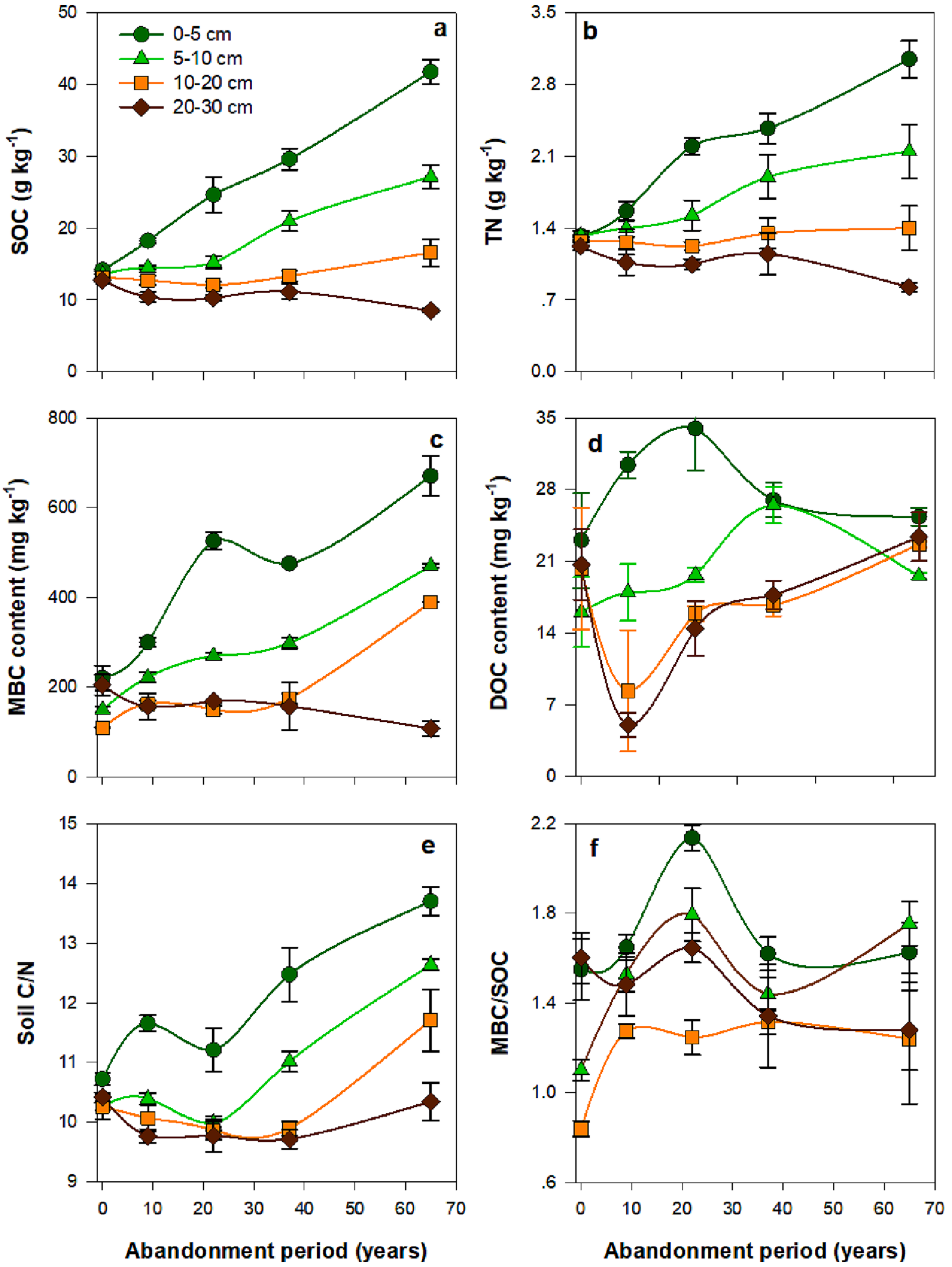

Fig. 1. Dynamics in soil properties (SOC, TN, MBC, DOC, Soil C:N, MBC:SOC) of the four topsoil depths until 30 cm across a chronosequence of abandoned soils. Values are the means ± standard error (n = 4). SOC, soil organic carbon; TN, total nitrogen; MBC, microbial biomass; DOC, dissolved organic carbon. The MBC/SOC ratio is presented in % of SOC content.

moisture was calculated as: soil moisture = $(W_{fresh} - W_{air\text{-}dried})/W_{fresh} \times 100$. Where $W_{fresh} - W_{air\text{-}dried}$ represent the weight of fresh and air-dried soils.

2.3. Microbial biomass carbon

The MBC was determined by the fumigation-extraction method as described by Vance et al. (1987). Briefly, 7 g of fresh samples were fumigated with $CHCl_3$ for one day in a desiccator. After removing the $CHCl_3$, soluble C from fumigated and non-fumigated samples were extracted with K_2SO_4 (0.5 M, 30 mL) by shaking for 1 h with 20 rpm via orbital shaker. Extracts were filtered and soluble C and N were measured using a Multi N/C 2100s (Analytik Jena, Germany). The dissolved organic C (DOC) corresponded to the soluble C was extracted by K_2SO_4 in the non-fumigated extract. The difference in soluble C content between fumigated and non-fumigated samples was calculated as MBC using a factor of 0.45.

2.4. Enzyme assay

The enzyme activities were determined using the fluorogenically labelled method (Marx et al., 2001; Zhou et al., 2020), which was described in details for three C-acquiring enzymes (β-glucosidase, BG; β-cellobiohydrolase, CBH, and β-xylosidase, XYL), two N-acquiring enzymes (leucine-aminopeptidase, LAP; chitinase, NAG), and one P-acquiring enzyme (acid phosphatase, AP). The enzyme activity was

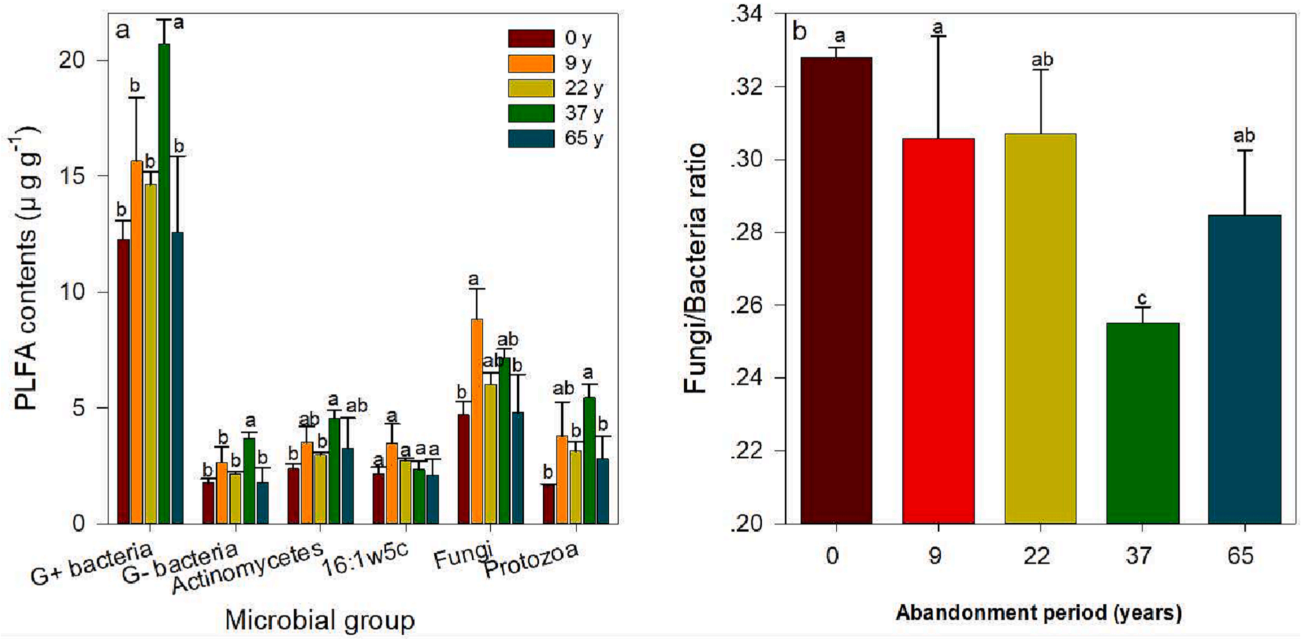

Fig. 2. Phospholipid fatty acids (PLFA) analysis of the microbial community in top 0–5 cm soil (a) and fungi-to-bacteria ratios (b) across a chronosequence of abandoned soils. Values are the means ± standard error (n = 4). Lowercase letters are used to show significant differences with abandonment time ($p < 0.05$) based on one-way ANOVA followed by LSD test.

analyzed by two synthetic fluorescent indicators: 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUF). Briefly, 0.5 g soil (dry weight equivalent) was suspended in 50 mL sterile water shook for 30 min and dispersed with an ultrasonic disaggregator for 2 min using low-energy sonication (50 J s^{-1}). An equal amount of 50 μL soil suspension was pipetted into 96-well microplates, and then 50 μL buffer and 100 μL of the substrate at a concentration of 2, 5, 10, 20, 50, 100, and 200 μmol substrate g^{-1} soil were added. After substrate addition, the microplates were fluorometrically determined at an excitation wavelength of 355 nm and an emission wavelength of 460 nm at 0, 30, 60, and 120 min. The enzyme activities are expressed as nmol g^{-1} dry soil h^{-1}.

We normalized the enzyme activities showing similar functions, such as C-acquiring (BG, XYL, and CBH), N-acquiring (NAG and LAP), as well as P-acquiring (AP) (Ma et al., 2022; Jia et al., 2022) as follows:

$$C\text{-}acq = (BG + \mathrm{XYL} + \mathrm{CBH})/3$$

$$\mathrm{x} = (\mathrm{BG} + \mathrm{XYL} + \mathrm{CBH})/(\mathrm{BG} + \mathrm{XYL} + \mathrm{CBH} + \mathrm{AP})$$

$$\mathrm{y} = (\mathrm{BG} + \mathrm{XYL} + \mathrm{CBH})/(\mathrm{BG} + \mathrm{XYL} + \mathrm{CBH} + \mathrm{LAP} + \mathrm{NAG})$$

$$Vector\ length = SQRT(x^2 + y^2)$$

$$Vector\ angle = DEGREES\ (ATAN2(x, y)$$

Where vector length represents the enzyme activity towards C acquisition relative to N and P (Ma et al., 2022). The higher vector length, the higher microbial C limitation; the larger vector angle indicates a greater microbial P limitation, while lower vector angle represents stronger microbial N limitation. Generally, vector angles > 45° implies P limitation whilst vector angle < 45° implies N limitation.

2.5. PLFA analysis

Phospholipid fatty acids (PLFA) were determined based on the protocol described by Gunina and Kuzyakov (2014). Briefly, 6 g of soil were extracted with a 25 mL one-phase mixture of chloroform, methanol and 0.15 M aqueous citric acid with a ratio of 1:2:0.8 (v/v/v, pH 4.0). Chloroform and citric acid were added to the extract to achieve a phospholipids separated from neutral lipids and glycolipids by solid phase extraction. The fatty acid methyl esters (FAMEs) were then purified by extraction with hexane. PLFAs i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, 20:1ω9 were referred to Gram-positive bacteria (G+); cy17:0, cy19:0, 16:1ω7, and 18:1ω7, 18:1ω9 were viewed as Gram-negative bacteria (G+); 16:1ω5 and were indicated as arbuscular mycorrhizal and fungi; the sum of10Me16:0, 10Me17:0 and 10Me18:0 were referred to actinobacteria; 20:4ω6c was viewed as prorozoa (Frostegård et al., 1993; Yao et al., 2012; Gunina et al., 2017; Zhou et al., 2022b). Total bacterial biomass was calculated as the sum of G+, G-, and actinobacteria.

2.6. Statistics

All statistical analyses were conducted using the open source software R version 3.2.0. Normality of residuals and variance homogeneity were tested with Shapiro-Wilk and a Bartlett test, respectively. Two-way ANOVA was applied to characterize the effects of abandoned age and soil depth. When significant effects were identified, one-way ANOVA followed by LSD test was performed ($p < 0.05$). Redundancy analysis was used to identify the contributions of soil properties to soil enzymes using the R package "Vegan". Data are expressed as mean of four replicates ± standard error (SE). If not specified, the discussed differences are significant at a p-value < 0.05.

3. Results

3.1. Soil organic carbon and microbial biomass carbon

The content of SOC in 0–10 cm increased from 15 g C kg^{-1} in arable soil to 30 g C kg^{-1} in soil after 37 years of restoration, while difference in SOC content in the deeper soil layer (10–30 cm) were absent (Fig. 1a). After abandonment, TN content in the 0–5 cm soil increased by 1.2 to 1.7 times compared to arable soil ($p < 0.05$, Fig. 1b). The MBC content in the 0–5 cm soil was 2-folds larger in abandoned soil after 22 years compared with arable soil ($p < 0.05$, Fig. 1c), and then it remained at similar levels between abandoned soil of 37 years and forest soil. MBC content in the 5–20 cm soil also increased significantly after

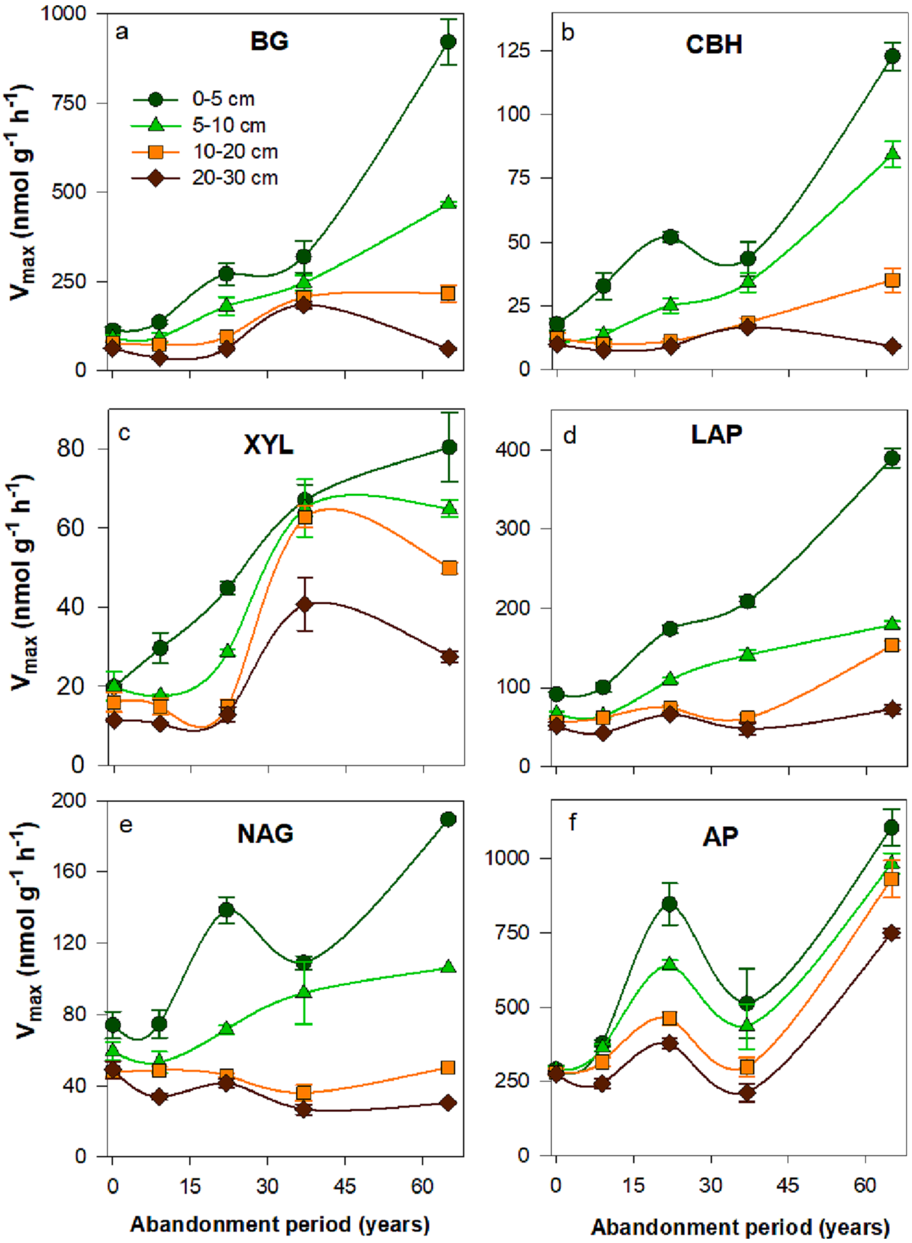

Fig. 3. Dynamics of enzyme activities of the four topsoil until 30 cm across a chronosequence of abandoned soils. BG, β-glucosidase; CBH, β-cellobiohydrolase; XYL, β-xylosidase; LAP, leucine-aminopeptidases (LAP); NAG, chitinase; AP, acid phosphatase. Values are the means ± standard error (n = 4).

abandonment of cropland ($p < 0.05$, Fig. 1c), while it remained stable in the 20–30 cm soil. DOC content in 0–5 cm soil was the highest in the 22-year abandoned soil among all abandoned sites (Fig. 1d). The C/N ratio in the 0–5 cm soil increased from 11 in arable soil to around 13 in abandoned lands, whereas it was lower than that in soil of a 65 years forest (~14) (Fig. 1e). The MBC/SOC ratio in the top 0–20 cm soils increased from arable to abandoned soil of 22 years old, and it was decreased to 1.5 on average in soil after 37 years of abandonment (Fig. 1f).

3.2. Microbial community composition

In the top 0–5 cm soil, the abundance of G+ bacteria, G− bacteria, fungi, actinomycetes, and protozoa were higher by 68%, 21%, 27%, and 87% in abandoned soil of 37-years old compared to arable soil ($p < 0.05$, Fig. 2a). Whilst the abundance of fungi was 1.1 times higher in soil after 9 years of abandonment than in arable soil ($p < 0.05$). The fungi and bacterial abundance were similar in arable and forest soils. In soil after 37 years of abandonment, the ratio of fungi and bacteria was the lowest (around 0.25) in the chronosequence ($p < 0.05$, Fig. 2b).

3.3. Soil enzymes activities and stoichiometry

The enzyme activities decreased with the soil depth and followed the order: 0–5 cm > 5–10 cm > 10–20 cm > 20–30 cm, regardless of abandonment year and enzyme type (Fig. 3). The activities of all the tested enzymes were the highest in the top 0–20 cm of forest soil, while the lowest enzyme activities corresponded to arable soil. In the top 0–20 cm soil, the activity of BG, XYL, and LAP increased with the abandonment year ($p < 0.05$, Fig. 3), whilst CBH, NAG, and ACP activities showed the highest value in soil after 22 years of abandonment.

Vector length in top 0–20 cm soil increased with the abandonment year and was the highest in abandoned soil of 37-yrs old ($p < 0.05$, Fig. 4a), suggesting a microbial C limitation. In the deeper 20–30 cm

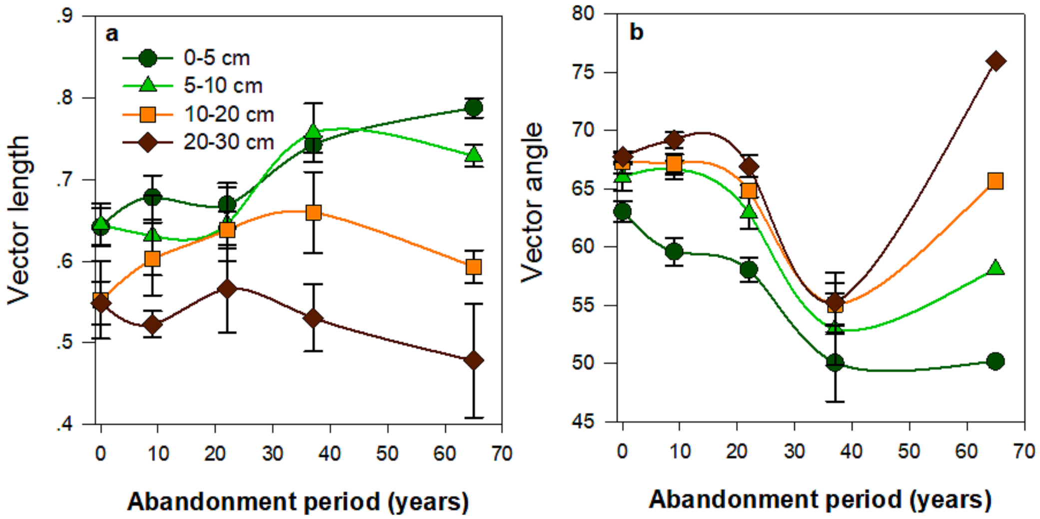

Fig. 4. Dynamics of the vector length (a) and angle (b) of the four topsoil depths until 30 cm across a chronosequence of abandoned soils. The vector length represents soil C limitation for microorganisms, while vector angle represents soil N and P limitation for microorganisms. Values are the means ± standard error (n = 4).

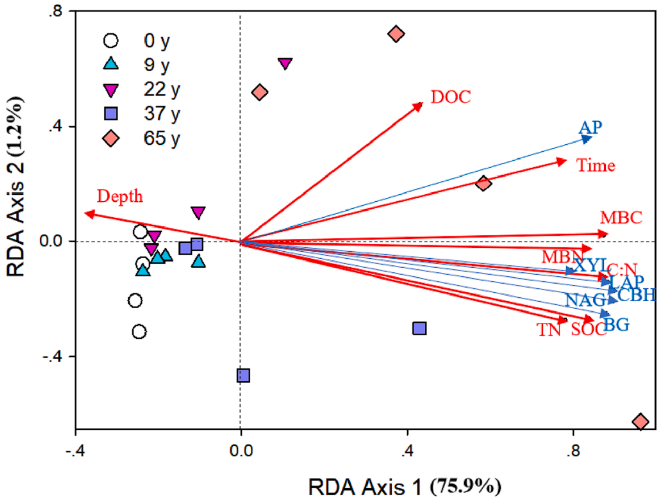

Fig. 5. Redundancy analysis (RDA) of enzyme activities and environmental variables at the soil 0–30 cm depth across a chronosequence of abandoned soils. The response variables were represented by red arrows and the effect variables were represented by blue arrows. BG, β-glucosidase; CBH, β-cellobiohydrolase; XYL, β-xylosidase; LAP, leucine-aminopeptidases (LAP); NAG, chitinase; AP, acid phosphatase; SOC, soil organic C; TN, total N; MBC, microbial biomass C; MBN, microbial biomass N; DOC, dissolved organic C.

soil, vector length remained stable during the self-restoration after cropland abandonment. All vector angles were larger than 45°, indicating a microbial P limitation regardless of soil depth and abandonment year (Fig. 4b). However, the vector angle in the 0–30 cm depth decreased with abandonment year from arable to 37-years abandoned soil ($p < 0.05$).

3.4. Relationship among soil biochemical properties, microbial biomass, and enzyme activity

The RDA analysis showed that RDA1 and RDA2 accounted for 75.9% and 1.2% of the variation in enzyme activities, respectively (Fig. 5). The main two axes captured 77.1% of the variability in the soil enzyme activities. Also, SOC was the dominant significant influencing factor for soil enzyme activity, which explained 51.1% of the variation in soil enzyme activity, followed by MBC ($p < 0.05$). Furthermore, there was a positive correlation between C-, N-, and P-related enzymes and SOC < MBC, and soil water content ($p < 0.05$, Fig. 6).

4. Discussion

4.1. Microbial biomass and community response to long-term self-restoration of soils

The MBC content increased during the self-restoration of arable soils in response to litter input, peaking in the 22-year-old abandoned soil, but did not reach up to the similar level in forest soil (Fig. 1c). As reported by Kalinina et al. (2018), the higher plant densities after 22 years of abandonment in the same area induced the large plant litter input and rhizodeposition, and reduced soil erosion, which supported the highest microbial biomass among soils of the chronosequence. This agreed with a higher microbial C use efficiency at early self-restoration stages (Sun et al., 2022), suggesting that the majority of immobilized C in the soil was used to form microbial biomass (Li et al., 2020). However, MBC in the top 0–5 cm soil declined despite an increase of SOC accumulation in the 37-years abandoned soil. This could be explained by that plant diversity and composition changed with abandonment period, which influenced the litter fall and root exudate composition (Fig. 7), thus increased a labile C fraction in the accumulated C (Kurganova et al., 2019). Given that soil microbial activity increases with nutrient availability (Xu et al., 2020), the lower SOC and TN contents led to a weak response of microbial activity to the abandonment year in the 20–30 cm soil depth (Fig. 1c).

The ratio between fungal and bacterial PLFAs in the surface soil decreased with abandonment year (Fig. 2b), indicating that bacteria become dominant at the later stages of restoration. This was contrary to Hu et al. (2022) who found that bacteria play an important role at the early stages of succession, whilst saprotrophic and mycorrhizal fungi dominant in the microbial community with restoration progressed. Since soil N content is a major factor influencing bacterial and fungal communities (Wang et al., 2021), improved soil nutrients were thus primarily responsible for the decreased abundance of fungal PLFAs (Figs. 1b, 2b), which belongs to oligotrophic groups and prefer nutrient-poor soils. Shifts from large populations of G− bacteria in surface soils to G+ bacteria could be explained by their preference for recalcitrant C in the litter over G− bacteria (Fig. 7). Given that actinomycetes are a metabolically versatile group of organisms that degrade high molecular

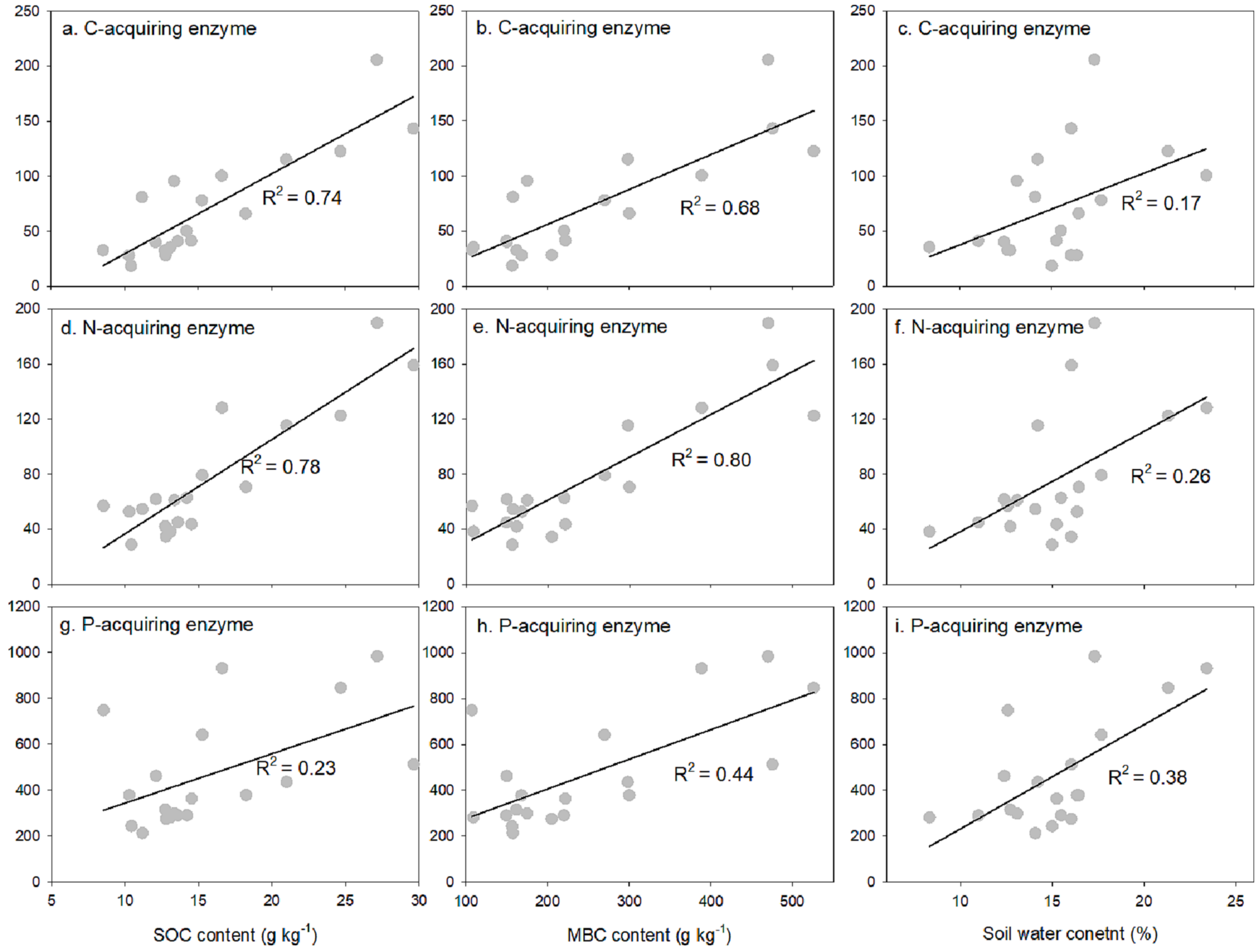

Fig. 6. Correlations between soil organic carbon (SOC) content, microbial biomass carbon (MBC), soil water content and enzymes related to C, N, P acquiring of the four topsoil until 30 cm across a Luvisol abandonment chronosequence. All regression lines are significant at $p < 0.05$. The C-acquiring enzyme is the sum of β-glucosidase; β-cellobiohydrolase, and β-xylosidase; the N-acquiring is the sum of chitinase and leucine-aminopeptidases; the P-acquiring enzyme is the sum of acid phosphatase. The unit of enzymes are expressed as nmol g^{-1} h^{-1}.

weight components of SOM including lignin and cellulose (McCarthy and Williams, 1992), the abundance of actinomycetes increased with abandoned year in out study site.

4.2. Enzyme activity and stoichiometric responses to long-term self-restoration of soils

After cropland abandonment, the majority of tested enzymes increased and peaked in the 22-year-old sites (Fig. 3). The enzyme activities increased due to the produced organics by the self-restored vegetation and their plant litter inputs (Singh et al., 2012). Greater organic inputs to soil over long periods increase SOC content (Fig. 1a) that provides C and energy and consequently increased microbial enzyme production (Nannipieri et al., 2012; Liu et al., 2023). The higher labile C increased microbial growth, which stimulates the utilization and assimilation of nutrients (i.e., N and P) via enzyme production (Zhou et al., 2021; 2022a). In addition, the nutrients could also serve as resources for microbial growth and subsequently increase microbial activities (Nayab et al., 2022). This was consistent with the increase of enzyme activities with SOC and MBC content (Fig. 6). A positive correlation between soil water content and enzyme activity (Fig. 6) also confirmed that the dominant role of soil moisture for biological function (Chen et al., 2017). This impact was an outcome of higher water holding capacity related to increased organic inputs from above- (litter) and below-ground (roots and rhizodeposition) since plant diversity increased from arable to forest soil, joint with the increased abundance of soil microorganisms (Xu et al., 2020). Enzyme biosynthesis and production is consequently stimulated as soil microbial community proliferates under more suitable physical and chemical environments (Keeler et al., 2009). However, the effect of cropland abandonment on enzyme activities was enzyme-specific. For example, β-glucosidase and β-cellobiohydrolase peaked in the 22-year-old sites, whilst β-xylosidase continued to increase with abandonment period (Fig. 3). At the late stage of post-agricultural recovery, the new vegetation is consisted mainly of shrub and trees, indicating a larger input of lignin, suberin and waxes (stable components) and less carbohydrates compared to grassland litter, which make litter more resistant to decomposition (Kögel-Knabner, 2017; Kalinina et al., 2018; Kurganova et al., 2019). Moreover, the C/N ratio of litter from forest was higher than on the former grassland (Thuille and Schulze, 2006), indicating a lower degradability. During forest development, plants with ectomycorrhiza or ericoid mycorrhiza replace plants with arbuscular mycorrhiza. Plants with these types of mycorrhiza are known for their slowly decaying litter (Cornelissen et al., 2001). These suggested that the higher recalcitrant litterfall after abandonment of arable soils favor the production and secretion of enzymes that decomposed the recalcitrant soil organic

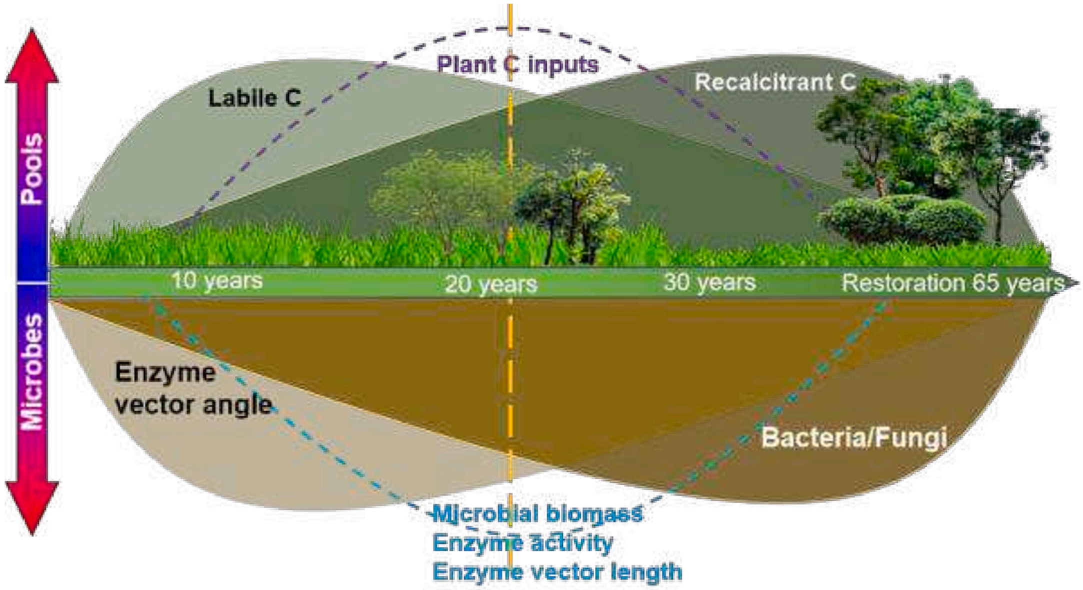

Fig. 7. Conceptual diagram of the dynamics of above and below-ground ecosystem properties wiithin a chronosequence of abandoned soils. Labile C and vector angle were the highest in the beginning of the cropland abandonment (9 year); plant C inputs, microbial biomass, enzyme activity, and vector length reached up to peak at 22 years since cropland abandonment; while recalcitrant C and the ratio of bacteria and fungi increased with the abandonment time.

matter, e.g. β-xylosidase (Fig. 3). It should be noted that we did not determine the amount of litter and root density as well as the quality of litter in different ecosystems in our case study. Therefore, ^{13}C and/ or ^{14}C chase labeling method should be applied to trace the photosynthetic C allocation belowground and quantify the amount of rhizodeposition. Furthermore, more studies should be conducted to evaluate the quantity and quality of litter and use the labeled litter to evaluate how much litter with different C:N ratio could be incorporated into microbial biomass, and consequently facilitate SOC accumulation with successional time.

Compared to surface soils (0–20 cm), the response of enzyme activities to cropland abandonment were slower in the deep soil layer (20–30 cm) (Fig. 4). Energy (C) availability would be the major driver of the vertical pattern of soil enzymes (Button et al., 2022), which could be evidenced by the RAD results that SOC was the dominant significant influencing factor for soil enzyme activity (Fig. 5), as well as the close relationship between MBC, SOC and soil enzymes (Fig. 6). Given that more than 50% of the roots were distributed in the upper 10 cm of the Ap horizon and the weighted average root biomass C declined with depth (Pausch et al., 2013), less amount of available C would be utilized by microorganism and consequently less enzymes were exuded in the deeper soil layers. The reduction in enzymes was also collaborated with the reduction in microbial activity and nutrients availability along soil depth (Fig. 1). This was ascribed to the decreased porosity and pore connectivity, which impedes the input of water, substrates and oxygen (Dungait et al., 2012).

The microbial C limitation in the top 0–20 cm increased in the soil of 37 years since cropland abandonment (Fig. 4a), suggesting an improve in the C and energy requirement (Fanin et al., 2016). This was consistent with Jiang et al. (2019) who documented that microorganisms in the top soil were limited by C in the initial revegetation period. Cropland abandonment increased microbial biomass and changed soil microbial community composition, resulting in a larger requirement for labile C and consequently an increased microbial C limitation (Fig. 4a). In line with, an increased activities of enzymes related to C acquisition was observed with post-agricultural restoration (Fig. 3). With more enzymes were secreted, larger C was required, which further stimulates microbial C limitation. Also, the alteration in plant litter quantity and quality (Fig. 7) as discussed above, inhibited SOM mineralization (Castro et al., 2010). This, in turn, increased microbial C limitation in the later stages of post-agricultural restoration. Furthermore, the vector angle decreased with cropland abandonment period (Fig. 4b), indicating that the microorganisms were more limited by N during self-restoration. This result was confirmed by the microbial activity in the rhizosphere was limited by N along the Hailuogou Glacier forefeld chronosequence (Li et al., 2020).

Noted, we did not observe stronger correlations between microbial community structure and enzyme activities in the top 0–5 cm soils. It is possible that, while the microbial community as a whole shifted, the relative abundance of enzyme producers did not. In this case the primary driver of depth trends in enzyme activities may be physiological shifts among enzyme producers. Finally, the lack of correlation between microbial community and enzyme activities may indicate other environmental mechanisms, such as enzyme immobilization and reduced turnover rates, are more important than community structure in accounting for the temporal distribution of enzymes.

4.3. Soil recovery after self-restoration

Soil recovered followed plant succession. The setting of a grass vegetation after abandonment caused a development of a well rooted Ah horizon in the top of the former plow horizon, accompanied by a reduction of aggregate sizes and bulk density (Kalinina et al., 2018). The subsequent secondary forest establishment caused the formation of an organic surface layer, and consequently inducing the development of a granular soil structure, which further reduced bulk density and aggregate size. These changes are in accordance with findings of other studies (e.g. Kalinina et al., 2015), and are expected to induced by the input of fresh organic residues which is linked to a greater diversity of plant communities, as well as higher activity and abundance of microbial communities (Lange et al., 2015). Given that microbial activity is typically C-limited, the organic matter such as glucose, cellulose and protein from the increased rhizodeposition could serve as energy for microbial growth, increase soil MBC (Fig. 7) and stimulate microbial metabolism C in the soil microbial community. Remarkably, greater enzyme activity and microbial biomass during self-restoration, indicating a stronger and faster C and nutrient turnover, and ultimately advance the process of multiple ecosystems functions. It seems that soils under 22 and 37 years after abandonment (such as greater diversity of plant species and higher abundance of perennial species) were effective in increasing microbial activity. The development towards natural soils was also observed in terms of SOC dynamics, which showed a recovery of SOC stocks during self-restoration. Hence, abandoning agricultural soils resulted in considerable C sequestration and confirms the strong mitigation of CO_2 emissions by abandonment.

5. Conclusions

Soil organic C accumulation during the 37 years of self-restoration strongly changed microbial activity and composition. The microbial biomass in the surface 0–10 cm soil increased by 1.0–2.5 times and peaked in 22 years post-agricultural soil. PLFA analysis indicated that bacteria rather than fungi dominate in the microbial community in the post-agricultural soil. Microbial biomass and enzyme activities increased faster than SOC accumulation during the early stages of self-restoration (less than 22 years). This could be explained by the large input of fresh plant residues into the topsoil. Specially, enzymes involved in C cycling increased more than N- and P-related enzymes, indicating higher microbial demand for C than for nutrients up to 37 years after abandonment. This was caused by the accumulation of recalcitrant compounds in plant litter at the later stages of self-restoration. Changes in microbial and enzyme activities in the soil deeper than 20 cm were weak. Consequently, cropland abandonment has a profound impact on the topsoil microbial environment, and therefore, it could have a great impact on nutrient cycling compared to the subsoil. Stoichiometric analyses of enzyme activities suggested that microorganisms are C and N limited along the post-agricultural restoration. Overall, this study highlights that soil recovery after degradation during agricultural use improves environmental conditions, and contributes to higher C and N availability, ultimately accelerates biogeochemical cycles and accelerates ecological functions (enzyme production and nutrient cycling).

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116496.

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