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Article

Changes in Soil Microbial Community along a Chronosequence of Perennial Mugwort Cropping in Northern China Plain

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Abstract: Perennial cropping play vital roles in regulating soil carbon sequestration and thus mitigating climate change. However, how perennial cropping affects soil microbial community remains elusive. Using a field investigation, this study was conducted to examine the effects of mugwort cropping along a chronosequence (that is, wheat-maize rotation, 3-year, 6-year, and 20-year mugwort cropping) on soil microbial community in temperate regions of Northern China. The results showed that the highest total, actinomycetes, and fungi PLFAs were found in the 3-year mugwort cropping soils. All PLFAs of microbial groups were lowest in the 20-year mugwort cropping soils. All of the three cropping years of mugwort increased network complexity of soil microbial community. Changes in total nitrogen and phosphorus content as well as the ratio of ammonium nitrogen to nitrate nitrogen could be primarily explain the variations in soil microbial community along the mugwort cropping chronosequence. Our observations highlight the contrasting impacts of soil microbial community to short-term and long-term mugwort cropping compared to conventional rotations and would have critical implications for sustainable agricultural management under perennial cropping in temperate regions.

Keywords: mugwort; perennial cropping; conventional rotations; sustainable agriculture; soil microbial community

1. Introduction

Soil microorganisms play critical roles in regulating key and fundamental ecosystem processes, such as decomposition of organic matter, nutrient mineralization and cycling, as well as plant nutrient uptake and growth (Guerra et al., 2021; Ali et al., 2022; Sokol et al., 2022). Evidence has shown that land-use types/intensities could have vital impacts on soil microbes (Venter et al., 2016; Siebert et al., 2019; Sünnemann et al., 2021; Huang et al., 2022). For example, conversion of grasslands to arable fields can decrease fungal diversity, which could result in loss of the microbial genetic resources and thus reduction of soil fertility (French et al., 2017). In addition, compared to natural ecosystems, croplands have lower bacterial diversity, with consequently regulates carbon dioxide efflux from soil of agro-pastoral ecotone on the Qinghai-Tibetan Plateau (Huang et al., 2022). As an important land-use type, perennial cropping has been demonstrated to increase soil carbon sequestration and mitigate climate change (Glover et al., 2010; Ledo et al., 2020; Zhu et al., 2021). Evidence has shown that perennial cropping can increase soil microbial biomass (Alagele et al., 2020; Wang et al., 2021). Compared to annual crops, perennial crops could enhance soil microbial community richness in a South European agricultural area (Cattaneo et al., 2014). However, the underlying mechanisms associated with the effects of perennial cropping on soil microbial community structure and composition remain to be elusive, especially at the broader and long-term scales.

Increasing evidence has accumulated and highlighted the critical role of nutrient availability on soil microbial communities under different land-use types (Zeng et al., 2016; Zhou et al., 2017; Cline et al., 2018). For instance, sufficient nutrient availability could reduce soil microbial diversity in cropland (Zeng et al., 2016; Zhou et al., 2017). In addition, by exacerbating soil acidification or carbon limitation, high nitrogen supply can also decrease soil microbial biomass and fungi-to-bacteria ratio in agricultural and natural ecosystems (Treseder, 2008; Zhou et al., 2017). However, the impacts of nutrient supply on soil microbial may change with the background nutrient status in ecosystems. Under high ambient nutrient availability, nitrogen enrichment decreases soil fungal richness, whereas increases it under low ambient nutrient availability (Moore et al., 2021). The finding indicates that responses of soil microbial community to nutrient availability may be nonlinear (Zhou et al., 2012; Guo et al., 2017). As another important nutrient for microbes, soil phosphorus availability could also mediate the abundance of diversity arbuscular mycorrhizal fungi (AMF) and their symbiotic function in cropping systems, suggesting that proper phosphorus levels in soil can benefit the AMF community and thus for productivity and sustainability of agricultural ecosystems (Smith et al., 2011; Gosling et al., 2013; Qin et al., 2020). In addition to nutrient availability, soil nutrient types (such as ammonium nitrogen (NH_4^+-N) vs. nitrate-nitrogen (NO_3^--N)) may also have divergent impacts on soil microbial community. For example, soil gram-positive and negative bacterial biomass is regulated by soil NH_4^+-N and NO_3^--N, respectively, and total microbial biomass is affected by NH_4^+-N rather than NO_3^--N in a forest ecosystem (Feng et al., 2022). In contrast, soil microbes prefer to use NO_3^--N in agricultural systems (Inselsbacher et al., 2010).

Low soil pH induced by nitrogen enrichment has also been demonstrated to decrease soil microbial growth and thus microbial biomass (Averill and Waring, 2018). Specifically, most *Acidobacteria* are acidophilic bacteria and their abundances rely on soil pH, indicating that changes in soil pH may select some microbial taxa (Rousk et al., 2010). This can lead to shifts in soil microbial community composition in agro-ecosystems (Lauber et al., 2008; Joa et al., 2014; Zhalnina et al., 2015). As an important cropping type, perennial cropping could have impacts on the above factors (Bnen et al., 2011; Ledo et al., 2020; Zhu et al., 2021) and thus have the potential to affect soil microbial community structure and composition. However, direct experimental evidence on the relative importance of various soil properties to changes in soil microbial community along a chronosequence of perennial cropping is scarce. Moreover, long-term continuous perennial cropping may not always benefit soil health due to continuous cropping obstacles (Chen et al., 2020; Wang et al., 2020; Tan et al., 2021). Therefore, it is urgently needed to explore the response of soil microbial community to short- and long-term perennial cropping, which is vital for the development of sustainable agricultural production.

As an important perennial medicinal plant, the demand for mugwort (*Artemisia argyi*) is increasing in China due to its medicinal value, and the cropping area is thus increasing in recent years, especially in Henan Province, a dominant agricultural region of North China Plain. There is reduced tillage during mugwort cropping compared to the conventional cropping and this can lead to enhanced soil carbon sequestration potential in the short-term (unpublished data). However, to the best of our knowledge, few studies have been conducted to explore the effects and underlying mechanisms of spatio-chronological perennial mugwort cropping on soil microbial community, which limits our ability to better understand and develop the sustainable agricultural under perennial cropping. In this study, we selected a chronosequence (0, 3, 6, and 20 years) of perennial mugwort cropping in the Northern China Plain to examine the scientific questions: 1) how the perennial mugwort cropping affects soil microbial biomass and composition compared to conventional cropping; 2) which factors regulate soil microbial biomass and composition under the perennial mugwort cropping chronosequence?

2. Material and Methods

2.1. Site description and sample collection

This study was conducted in Tangyin County (35°45'-36°01' N, 114°13'-114°42' E), Anyang, Henan, where is one of the origins of mugwort, a genuine herb. This region has a warm temperate continental monsoon climate, with long-term mean annual temperature and precipitation was 13.4 °C and 582.0 mm, respectively. The soil is classified as cinnamon soil according to the Chinese soil classification system. The surface soil contained organic matter and total nitrogen (N) content 16.7 mg/g and 1.07 mg/g, respectively. With the development of the traditional Chinese medicine industry, parts of croplands were gradually converted from maize-wheat rotation to perennial mugwort cropping in Tangyin County in recent years, which provides the opportunity to assess the effects of the chronosequence of perennial mugwort cropping on soil microbial community.

We identified and selected a chronosequence (continuous maize-wheat rotation (Control-Y0), perennial mugwort cropping for 3 (Y3), 6(Y6), and 20 years(Y20)) of perennial mugwort cropping in this study region. 25 kg compound fertilizers were applied per year in each of the four cropping chronosequences. In each chronosequence period, three blocks were randomly established. Three 20 cm depth (0-10 cm, 10-20 cm) cylindrical holes were excavated using a soil auger (5 cm in diameter) in the plots. Soil samples were passed through a 0.25 mm sieve and roots were collected then oven-dried at 105 °C for 48 hours. Then, soil samples were divided into two subsamples. One subsample was stored at 4 °C for measuring soil physicochemical properties. Another subsample was stored at -20 °C for measuring soil extracellular enzymatic activities and PLFAs.

2.2. Soil chemical property measurements and PLFA analysis

Soil total N were measured by an elemental analyzer with a dry combustion method (Miao et al., 2020, Vario MAX CN, Elementar Co., Germany). The total phosphorus (P) content was determined by H_2SO_4-$HClO_4$ digestion and then P molybdenum blue colorimetric analysis (Murphy and Riley, 1962; Guo et al., 2021). The concentrations of ammonium (NH_4^+) and nitrate-nitrogen (NO_3^-) were extracted with 2M KCl solution and measured by a flow injection analyzer (SAN-System, Netherlands). Soil pH was measured with a combination glass-electrode [soil:water = 1:2.5 (W/V)]. The results of soil properties along the chronosequences of mugwort cropping were shown in another manuscript which is under review.

Soil microbial community was analyzed with PLFA analysis. Lipids were extracted from 8 g freeze-dried soil using a mixture of chloroform-methanol-phosphate buffer (1:2:0.8 v/v/v) and separated into phospholipids, glycolipids, and neutral lipids using an LCSi SPE silica column. The phospholipids were subjected to mild alkaline methanolysis, and the fatty acid methyl esters were identified on a gas chromatograph fitted with the MIDI Sherlock Microbial Identification System. Methyl nonadecanoate fatty acid (19:0) was used as the internal standard for quantifying the abundances of individual fatty acids for each sample by converting GC peak areas to nmol·g^{-1} dry soil.

The PLFAs 16:1w7c, 18:1w7c, cy19:0w7c, cy17:0w7c were regarded as gram-negative bacteria (GN), a15:0, a17:0, i15:0, i16:0, i17:0, and i17:1w9c were considered as gram-positive bacterial (GP). Two fatty acids, 18:1w9c and 18:2w6c, were biomarkers for fungi and 16:1w5c was used to represent arbuscular mycorrhizal fungi (AMF). 10Me16:0, 10Me18:0, and 10Me18:1w7c were actinomycetes biomarkers. The ratio of fungi to bacteria (F:B ratio, the sum of GP, GP, and unspecific bacteria biomarkers 16:0 and 18:0) was also calculated.

2.3. Statistical analyses

Two-way ANOVAs were used to explore the effects of soil depth and cropping year on all the variables included in the study. In addition, changes in soil chemical properties among the four cropping types were assessed using one-way ANOVA with Ducan multiple comparisons. Principal components analysis (PCA) was performed to explore microbial variation among the four cropping types using OriginPro 2022 (OriginLab Corporation, Northampton, MA, USA). The co-occurrence network was constructed and visualized by Gephi (v.0.9.2). The correlations among variables were explored by the Pearson correlation method. Significant differences were evaluated at the 0.05 probability level. Then, we used Random Forest (RF) models to partition relative influences of NH_4^+, NO_3^-, NH_4^+/NO_3^-, TN, TP, and pH on microbial variation. Statistical analyses were performed using SAS 8.0 (SAS Institute Inc., Cary, NC, USA) and R v.4.1.1 (R Development Core Team). GraphPad Prism 9.0 (GraphPad Inc., San Diego, California, USA) were used to plot the graphs.

3. Results

3.1. Soil microbial biomass and composition

There were significant differences in total PLFAs between the two soil depths (Table 1, $P < 0.01$). Similar differences in bacteria, fungi, gram-negative bacteria, and arbuscular mycorrhizal fungi (AMF) PLFAs were also found between the two soil depths across the four cropping types (Table 1, all $P < 0.01$). In addition, all PLFAs showed variations among the four cropping types across the two depths (all $P < 0.05$). The highest total, actinomycetes, and fungi PLFAs were found in the 3-year mugwort cropping soils. No differences in gram-positive and gram-negative bacteria PLFAs were detected among the conventional rotation, 3-year and 6-year mugwort cropping soils. All PLFAs were lowest in the 20-year mugwort cropping soils (Table S1, Fig. 1). Significant interactions between soil depth and cropping years on total, bacteria, and gram-negative bacteria PLFAs were detected (Table 1, all $P < 0.05$). In addition, the proportion of fungi under the 3-year mugwort cropping was higher than that under the other cropping types (Fig. S1). Similarly, the proportion of gram-negative bacteria under the 3-year mugwort cropping was 3.00% higher than that 20-year mugwort cropping. Although there were no differences in actinomycetes composition under the conventional rotation, 3-year and 6-year mugwort cropping, all of which showed higher actinomycetes composition than that under the 20-year mugwort cropping (Fig. S1).

Table 1. Results (*F*- and *P*-values) of two-way ANOVAs on the effects of soil depth (Depth), cropping year (Year), and their interactions on the total, bacteria, actinomycetes (ACT), fungi, gram-positive bacteria (GP), gram-negative bacteria (GN), arbuscular mycorrhizal fungi (AMF) PLFAs, and the ratio of fungi to bacteria (F:B ratio). The bold numerals indicate the significance at $P \leq 0.05$.

Variations	Depth		Year		Depth×Year	
	F	*P*	*F*	*P*	*F*	*P*
Total	11.44	**0.004**	9.57	**<0.001**	3.52	**0.04**
Bacteria	14.30	**0.002**	11.29	**<0.001**	3.18	**0.05**
ACT	2.86	0.11	18.82	**<0.001**	0.69	0.57
Fungi	9.90	**0.006**	3.36	**0.04**	1.53	0.25
GP	3.84	0.07	5.35	**0.01**	2.53	0.09
GN	24.41	**<0.001**	15.13	**<0.001**	3.51	**0.04**
AMF	10.47	**0.005**	6.97	**0.003**	3.03	0.06

F:B ratio	3.43	0.08	0.83	0.50	0.57	0.64

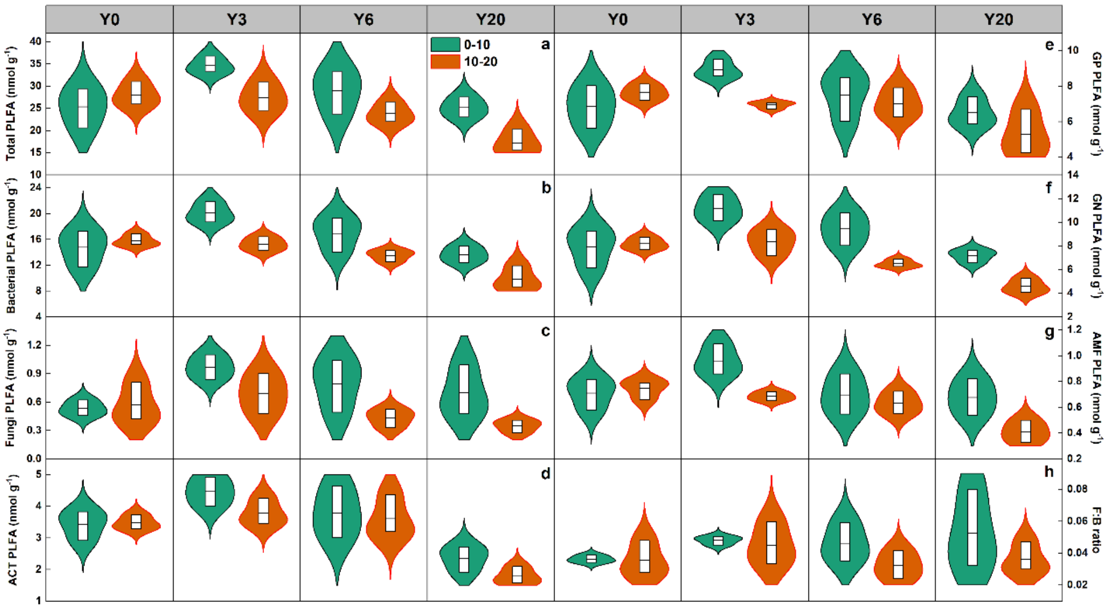

Figure 1. Total (a), bacterial (b), fungi (c), actinomycetes (ACT, d), gram-positive bacteria (GP, e), gram-negative bacteria (GN, f), arbuscular mycorrhizal fungi (AMF, g), and the ratio of fungi to bacteria (F:B ratio, h) under different cropping years of mugwort. Y0: continuous maize-wheat rotation; Y3: mugwort cropping for 3 years; Y6: mugwort cropping for 6 years; Y20: mugwort cropping for 20 years.

3.2. Soil microbial network analysis

PLFA-based soil microbial composition showed significant variations in the two soil depths (0-10cm and 10-20cm, Fig. 2a) and different cropping year (Fig. 2b). The first and second principal components accounted for 75.80% and 13.70% of the variations, respectively. To describe the co-occurrence patterns of soil microbial communities, the ecological networks were conducted. The results revealed that 32 nodes and 102 edges under the conventional rotation, with the node degree and average path length of 3.29 and 2.10, respectively. The edge of the conventional rotation was lower than that of the 3-year (141), 6-year (141), and 20-year mugwort cropping (127). In addition, node degrees were 4.58, 5.04, and 4.23 under the 3-year, 6-year, and 20-year mugwort cropping, respectively (Fig. 3).

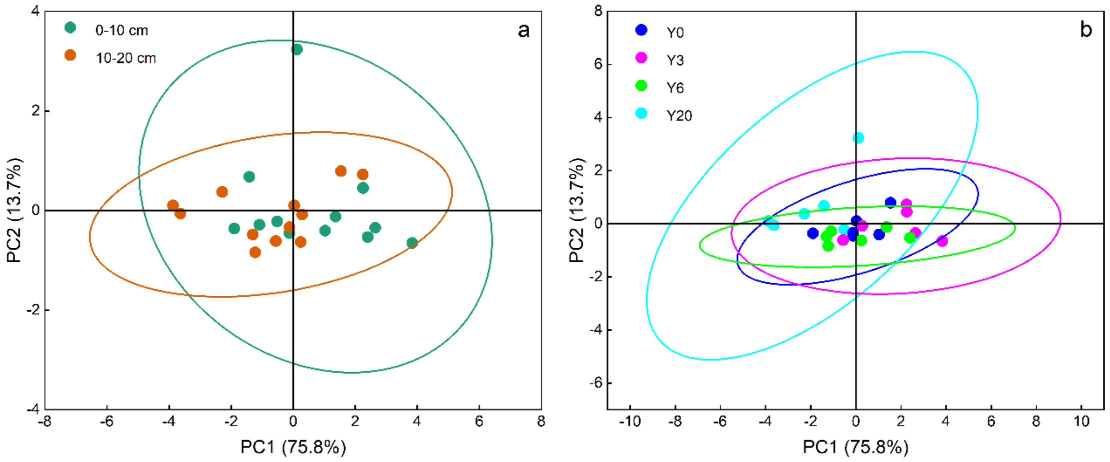

Figure 2. The principal components analysis (PCA) representing the variation of microbial community among different soil depths (a) and cropping years (b). Each datapoint represents the microbial community of soil sample in each plot. Solid circles represent the 95% confidence intervals. See Fig. 1 for abbreviations.

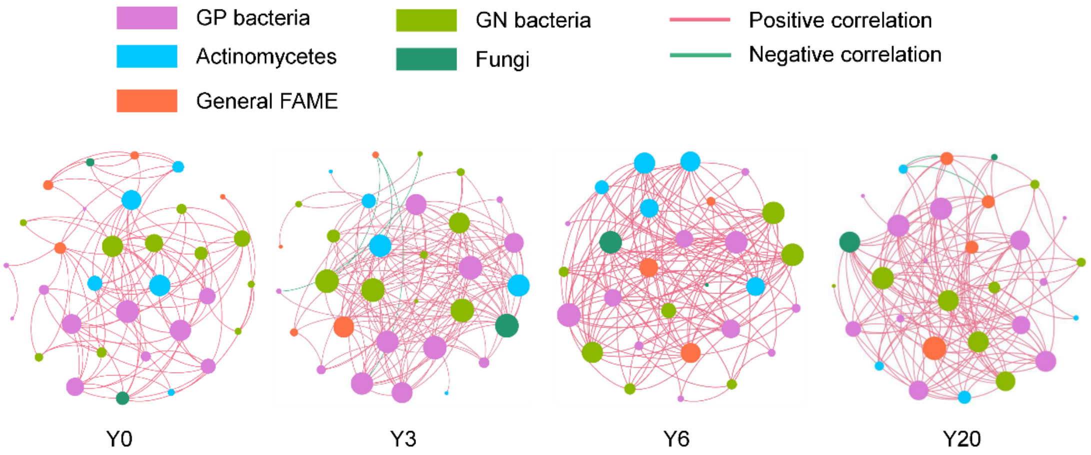

Figure 3. Network analysis of co-occurring PLFAs under different mugwort cropping years. Nodes with different colors represent the individual PLFA biomarkers. Solid red and blue lines represent positive and negative correlations, respectively. General FAME: unspecific biomarkers. See Fig. 1 for abbreviations.

3.3. Driving factors of soil microbial community

Bacteria biomass increased with increasing soil NH_4^+-N ($R^2 = 0.20$, $P = 0.03$), total N ($R^2 = 0.56$, $P < 0.001$) linearly, whereas decreased with increasing soil pH ($R^2 = 0.23$, $P = 0.02$). In addition, bacteria biomass showed quadratic relationships with NH_4^+/NO_3^- ($R^2 = 0.51$, $P < 0.001$) and total P ($R^2 = 0.41$, $P < 0.01$, Fig. 4). Similarly, quadratic relationships of ACT biomass with soil pH ($R^2 = 0.50$, $P < 0.001$), NH_4^+ ($R^2 = 0.39$, $P < 0.01$), NH_4^+/NO_3^- ($R^2 = 0.44$, $P < 0.01$), and total P ($R^2 = 0.71$, $P < 0.001$) were observed across the four cropping types. ACT biomass linearly increased with increasing total N ($R^2 = 0.72$, $P < 0.001$, Fig. 5). Fungi biomass showed quadratic dependences on NH_4^+/NO_3^- ($R^2 = 0.23$, $P = 0.06$), total N ($R^2 = 0.41$, $P < 0.01$) and P ($R^2 = 0.29$, $P = 0.03$, Fig. 6) only, respectively. Gram-positive bacteria biomass negatively and positively correlated with soil pH ($R^2 = 0.21$, $P = 0.02$) and total N (R^2

= 0.40, $P < 0.001$), respectively, whereas showed quadratic relationships with NH_4^+/NO_3^- ($R^2 = 0.50$, $P < 0.001$) and total P ($R^2 = 0.38$, $P < 0.01$, Fig. 7). There were significantly linear relationships of gram-negative biomass with soil NH_4^+ ($R^2 = 0.22$, $P = 0.02$) and total N ($R^2 = 0.61$, $P < 0.001$), whereas quadratic relationship with soil pH ($R^2 = 0.25$, $P = 0.05$), NH_4^+/NO_3^- ($R^2 = 0.47$, $P < 0.01$) and total P ($R^2 = 0.40$, $P < 0.01$, Fig. 8). Similar relationships of arbuscular mycorrhizal fungi biomass (AMF biomass) with soil pH ($R^2 = 0.29$, $P = 0.03$), NH_4^+/NO_3^- ($R^2 = 0.55$, $P < 0.001$), and total P ($R^2 = 0.35$, $P = 0.01$) were also detected, respectively (Fig. 9).

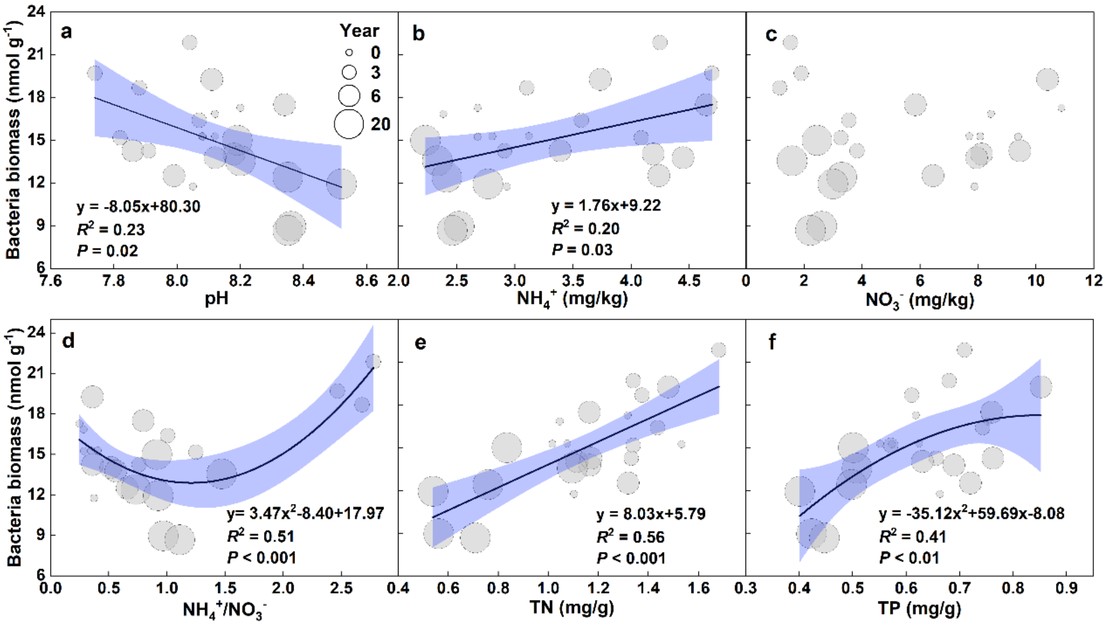

Figure 4. Relationships of bacteria biomass (nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

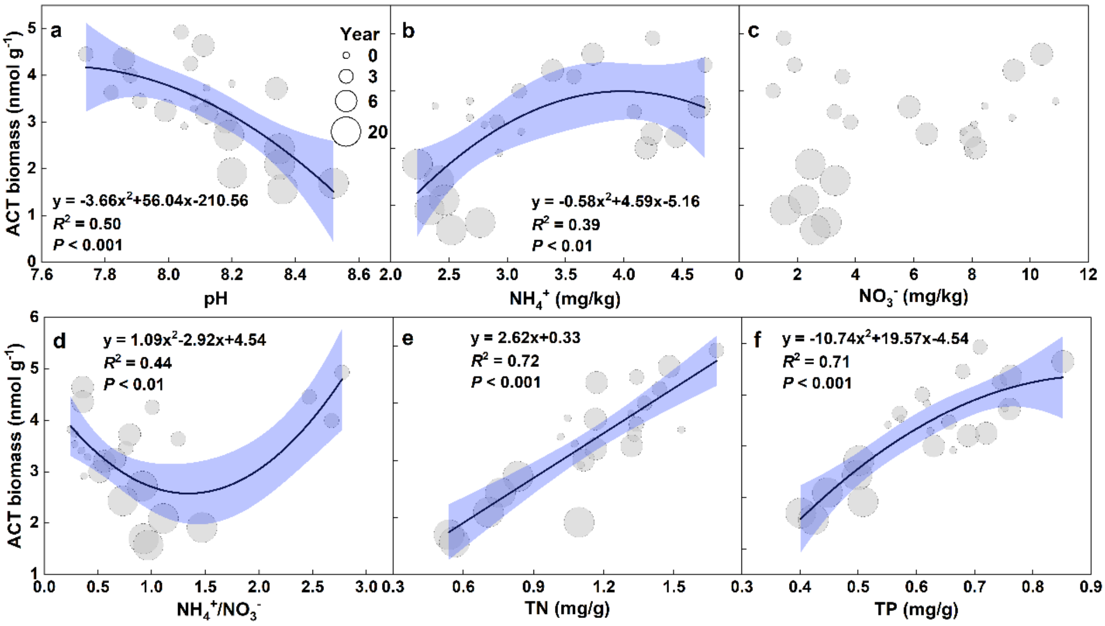

Figure 5. Relationships of actinomycetes biomass (ACT biomass, nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

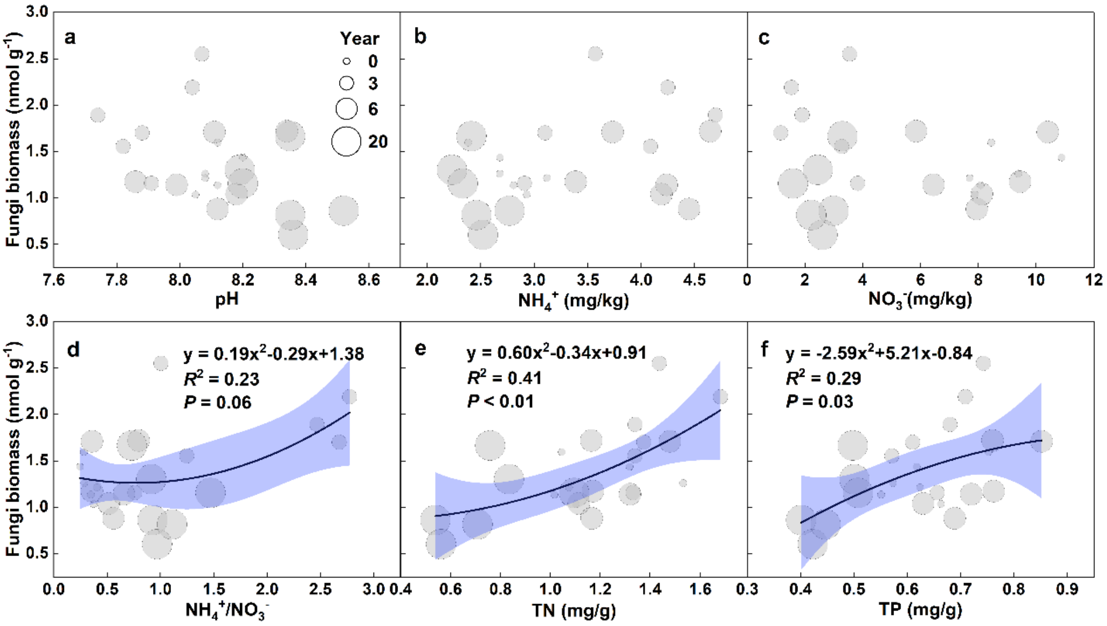

Figure 6. Relationships of fungi biomass (nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

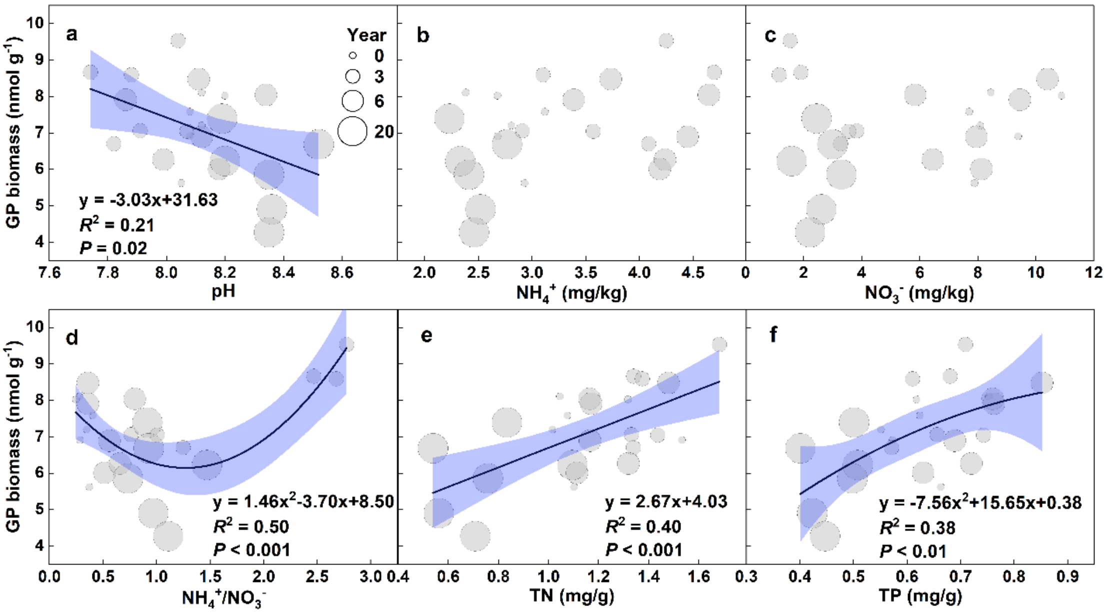

Figure 7. Relationships of gram-positive bacterial biomass (GP biomass, nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

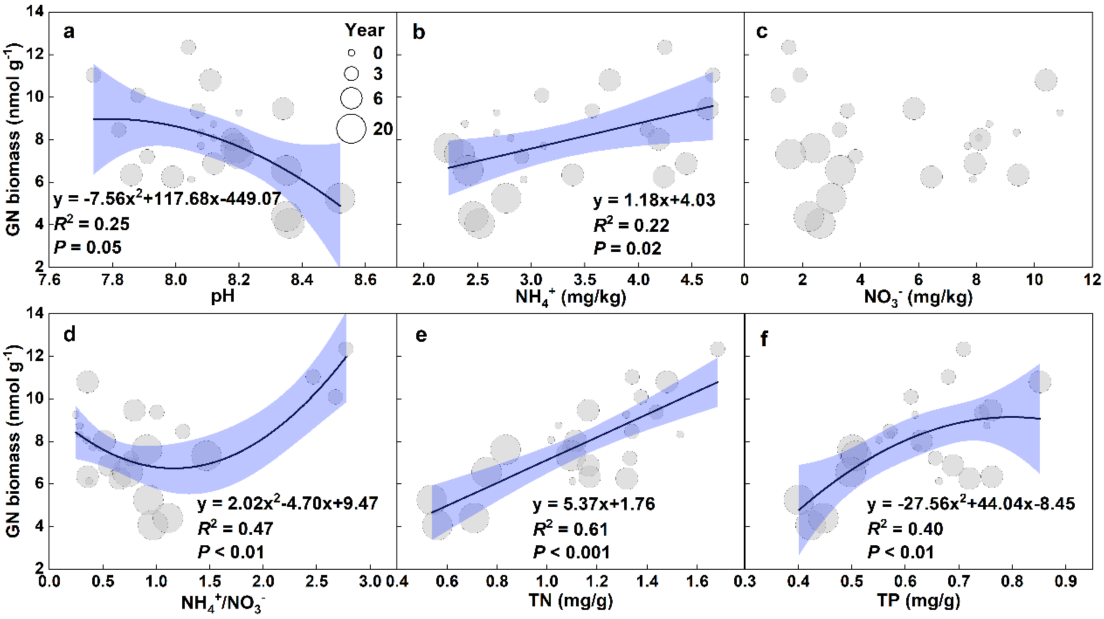

Figure 8. Relationships of gram-negative bacterial biomass (GN biomass, nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

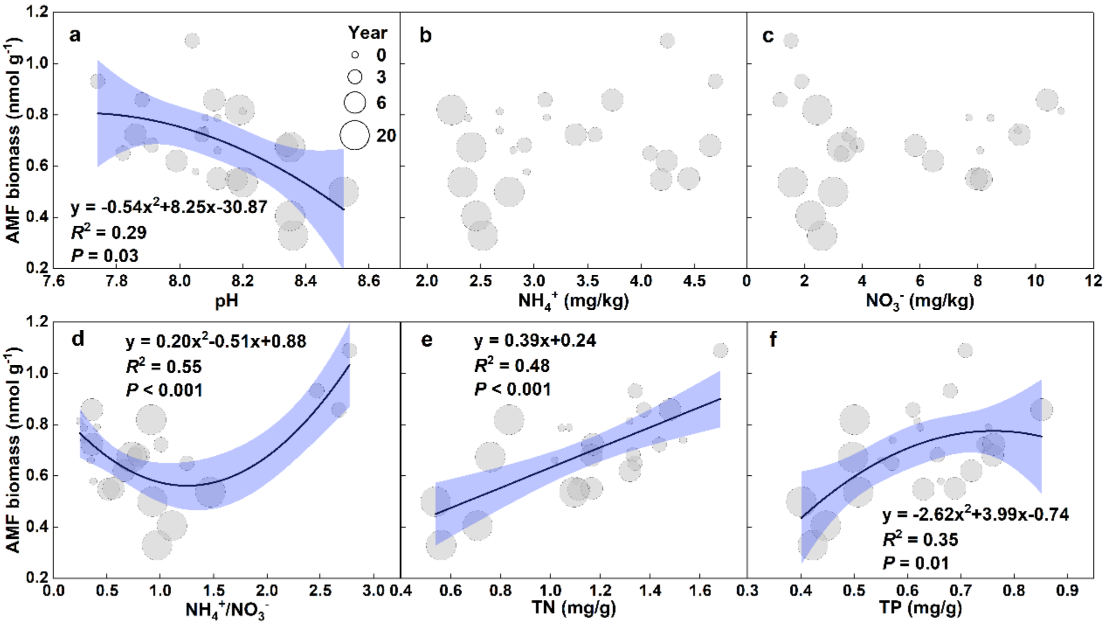

Figure 9. Relationships of arbuscular mycorrhizal fungi biomass (AMF biomass, nmol·g^{-1}) with soil properties. See abbreviations in Table 2.

The random forest model revealed that the ratio of NH_4^+ to NO_3^-, total N and P contributed more than the other sol properties to the changes in soil microbial community along the chronosequence of perennial mugwort cropping ($F = 6.49$, $P < 0.01$, Table 2). When analyzed by each PLFA, the ratio of NH_4^+ to NO_3^-, total N and P were also the most important factors to affect the bacteria ($F = 8.47$, $P < 0.001$), fungi ($F = 5.30$, $P < 0.01$), gram-positive ($F = 5.15$, $P < 0.01$), gram-negative ($F = 9.17$, $P < 0.001$), and

AMF PLFAs ($F = 5.56$, $P < 0.01$). By contrast, total N and P, as well as pH were more important than the other three soil properties to impact the ACT PLFAs ($F = 19.16$, $P < 0.001$, Table 2).

Table 2. Relative contributions (Increase in MSE, %) of soil properties to PLFAs based on Random Forest analysis. NH_4^+: soil ammonium-nitrogen content, NO_3^-: nitrate-nitrogen content, NH_4^+/NO_3^-: the ratio of NH_4^+ to NO_3^-, TN: total nitrogen content, TP: total phosphorus content.

Variables	Total		Bacteria		ACT		Fungi		GP		GN		AMF	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
	6.49	0.001	8.47	<0.001	19.16	<0.001	5.30	0.003	5.15	0.003	9.17	<0.001	5.56	0.002
pH	3.93		9.67		11.46		1.28		7.82		8.77		5.76	
NO_3^-	8.08		8.14		6.20		5.41		2.81		6.41		3.31	
NH_4^+	0.24		2.40		6.38		1.99		1.65		1.72		2.00	
NH_4^+/NO_3^-	13.37		13.29		6.51		9.61		13.47		12.05		12.61	
TN	14.59		16.63		16.72		13.23		9.67		18.47		9.64	
TP	13.70		11.53		16.96		10.41		8.65		11.20		9.22	

4. Discussion

4.1. Changes in soil microbial community along the chronosequence of perennial mugwort cropping

Previous studies have shown that perennial cropping can increase soil microbial biomass (Zaibon et al., 2016; Alagele et al., 2020). In this study, our results demonstrated that short-term mugwort cropping indeed increased soil microbial biomass (i.e., actinomycetes and fungal biomass, Fig. 1, Table S1). In contrast, long-term mugwort cropping decreased biomass of all microbial groups (Fig. 1, Table S1), which is inconsistent with those found in previous studies (Zaibon et al., 2016; Alagele et al., 2020). In this study, short-term mugwort cropping increased the complexity of co-occurring network among the microbial groups (Fig. 3), which indicates the stimulated microbial growth and activities under the short-term mugwort cropping. In contrast, although the complexity of co-occurring network under long-term mugwort cropping was greater than that under the conventional cropping, both plant biomass and soil nutrients were lower under the long-term mugwort cropping, which could restrain the growth and thus biomass of microbial groups through nutrient limitation (Hicks et al., 2019; Moore et al., 2021).

In addition, our finding showed that biomass of most microbial groups at the depth of 10-20 cm was lower than that at the depth of 0-10 cm. This observation could be attributed to three reasons. First, it has been demonstrated that root exudations associated with root biomass can supply nutrients for microbial growth (Moore-Kucera and Dick, 2008; Kramer et al., 2013). In this study, the lower root biomass of mugwort at the depth of 10-20 cm than that at the depth of 0-10 cm may supply less nutrients or root exudations for microbes, leading to the lower microbial biomass at the depth of 10-20 cm. The causal relationships of microbial biomass with root biomass could support this speculation (Fig. S2). Second, except for root exudations, soil nutrient availability also plays critical role in regulating microbial biomass and structure (Zhou et al., 2017; Zhang et al., 2018; Hicks et al., 2019; Moore et al., 2021). In fact, most of available nutrients at the depth of 10-20 cm were less than that at the depth of 0-10 cm (under review). The less nutrient availability could limit the soil microbial activities and growth (Cline et al., 2018; Moore et al., 2021). Third, different microbial community composition and structure may result in various competitions for nutrient among diverse microbial groups (Jiao et al., 2020). The PLFA-based soil microbial composition showed significantly different among the two soil depths may support the speculation (Fig. 2).

Our findings indicate that long-term mugwort cropping may not benefit for the sustainability of soil microbial biodiversity and functioning and that rotations should be considered when mugwort cropping for about 11 years (Fig. S3). Given the limited understanding of long-term perennial cropping on soil microbial community, our findings of the contrasting responses of soil microbial biomass and composition to short- vs. long-term cropping could have critical implications for the sustainable agricultural management in temperate regions.

4.2. Driving factors of soil microbial community along the chronosequence of perennial mugwort cropping

In the current study, soil properties decreased significantly under the long-term the mugwort cropping (Y20), especially soil NH_4^+, total N and P (under review). The findings may be attributed to three reasons. First, it has been demonstrated that long-term no-tillage management could lead to increased pathogens in soil (Wang et al., 2020). In this study, reduced tillage managements are applied during mugwort cropping, which may result in increased soil pathogens and thus the risk of root rot, with consequently suppress the nutrient absorption efficiency of mugwort roots. Second, tiller number increased significantly with the mugwort cropping year, which can lead to intra-specific competition for resources and nutrients, and thus have disadvantage for plant growth and development. Given the importance of fungi and AMF for nutrient uptake and plant growth, the lower fungi and AMF PLFAs (Fig. 1) combined with the decreased aboveground and root biomass (under review) provides further support for the above discussion. Third, as results of the above two potential reasons, soil nutrients cannot be absorbed by mugwort plants effectively, which may result in leaching and loss with precipitation, and consequently cause the significant reduction of soil nutrients under the long-term mugwort cropping.

Previous studies have shown that pH is an important factor in affecting microbial community in agro-ecosystems (Joa et al., 2014; Zhalnina et al., 2015; Francioli et al., 2016). However, the observations that negative relationships of microbial PLFAs with soil pH (except for fungi PLFAs) are inconsistent with those found in previous studies (Zhalnina et al., 2015; Ali et al., 2022). The difference may be resulted from the different soil types, crops, as well as managements. Mugwort has strong tillering ability and thus can efficiently abort soil NH_4^+ and NO_3^-, both of which are main drivers of soil acidification, and thus alleviate soil acidification process under fertilization in the short-term. In contrast, previous studies have demonstrated that most of soil microbes favor acidic or neutral environment (Yin et al., 2021; Ali et al., 2022), which could lead to the negative dependence of microbial PLFAs with soil pH in this study.

It has well been documented that soil nutrient availability have critical impacts on microbial community (Zhao et al., 2016; Zhang et al., 2018; Hicks et al., 2019; Moore et al., 2021). High soil available nutrient can supply diverse substrates and thus stimulate high microbial abundance and growth (Goldfarb et al., 2011; Cline et al., 2018). In this study, the findings of positive dependence of different microbial PLFAs with soil NH_4^+, total N and P are consistent with those found in previous studies. Interestingly, the observations of the causal dependence of changes in fungi and AMF PLFAs rather than bacteria PLFAs on soil NH_4^+ under the mugwort cropping chronosequence indicate that significant difference in nutrient use strategies between fungi and bacteria. In addition, all of microbial groups in this study are not sensitive to soil NO_3^- (Figs. 4-9), which suggests that farmers of mugwort cropping should consider NH_4^+ fertilizer rather than NO_3^- fertilizer to improve the soil microbial biomass and function. The evidence that quadratic relationships between microbial PLFAs and the ratio of NH_4^+ to NO_3^- provide further supports for above discussion. Moreover, to prevent nitrification associated with NH_4^+, nitrification inhibitor should be applied combined with NH_4^+ fertilizer. Our findings have critical implications for sustainable development of mugwort cropping in the future.

4. Conclusions

Using a filed investigation, we demonstrated that soil microbial biomass and composition showed various changes along the mugwort cropping chronosequence. The changes in soil properties, especially total nitrogen and phosphorus content, as well as the ratio of ammonium nitrogen to nitrate nitrogen explained the observations. In addition, the observations that the contrasting impacts of short-term and long-term mugwort cropping on soil microbial community compared to conventional rotations indicate that mugwort cropping has a key role in sustaining the abundance and structure of soil microbial communities, which may be advantage for preventing the degradation of the soil microbial community structure and function in the short-term. Our findings suggest that short-term perennial mugwort cropping can have potential to increase microbial biomass and rotations with other crops should be considered after short-term mugwort cropping to strengthen agricultural sustainability in temperate regions.

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