Plant Soil (2024) 496:641–656
https://doi.org/10.1007/s11104-023-06388-7

RESEARCH ARTICLE

Responses of the soil microbial community structure to multiple interacting global change drivers in temperate forests

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Received: 24 April 2023 / Accepted: 8 November 2023 / Published online: 18 November 2023

Abstract

Background The microbial community structure in forest soils is expected to change in response to global environmental change, such as climate warming and nitrogen deposition. Community responses to these environmental changes may further interact with the site's land-use history and understory light availability. Uncovering the relative importance of these global change drivers is crucial to understand and predict soil microbial communities' changes.

Methods A full-factorial *in situ* mesocosm experiment was conducted and the soil microbiota were analyzed by phospholipid fatty acid and neutral lipid fatty acid. The soils in the mesocosms were sampled from forests with different land-use history, and mesocosms contained typical forest understory plants. The mesocosms were exposed to experimental treatments of warming, nitrogen addition and subcanopy illumination.

Results Among the treatments, past land-use had the strongest effect shaping the microbial community structure. We found a significantly higher abundance of arbuscular mycorrhizal fungi and Actinobacteria in ancient forests. The soil microbial and plant communities were co-structured in ancient forests, but not in past-agricultural forests. Warming and nitrogen fertilization did not affect the soil microbial community composition, yet illumination resulted in slight changes in soil microbial composition.

Conclusions Our results underpin the role of land-use legacies in shaping soil microbial communities. The stronger plant-microbe linkages in ancient forest soils compared to post-agricultural secondary forest soils may contribute to a higher resilience against environmental changes. Our results advocate for more multifactor global change experiments that investigate the mechanisms underlying the potential effects of land-use legacies on plant-microbe relationships in forest.

Keywords Global change · land-use legacy · soil microbial community structure · plant communities · PLFA · climate change

Responsible Editor: Paul Bodelier.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-023-06388-7.

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Introduction

Global change drivers are reshaping the structure and processes of ecosystems, which influence the biodiversity and functioning of the ecosystems (Crowther et al., 2015; Rillig et al., 2019). In the face of rapid environmental change, soil microbiota are of importance to regulate ecosystem functioning at local or wider spatial scales (Crowther et al., 2015; Zhang et al., 2021). These microbial communities can respond to global change drives, such as atmospheric N deposition and climate change (Smith et al., 2009; Grimm et al., 2013). Besides, abrupt disturbances from natural dynamics or anthropogenic activities in the canopy may affect availability in resources, such as light (van Loon et al., 2014; Rui et al., 2016; Williams et al., 2020; Bochet et al., 2021). Soil microbiota community responses to environmental changes may further interact with legacies from previous changes, such as past land-use (Vellend et al., 2007; Perring et al., 2016). Elucidating the contribution of these global change drivers and land-use legacies to the soil microbial community is necessary to improve predictions of soil microbial community development, and to understand how ecosystem functions (e.g., carbon storage and nutrient cycling) may shift in response to multiple interacting global changes (Burke et al., 2011; Eisenhauer et al., 2012; Rillig et al., 2019; van der Plas, 2019).

Soil microbiota, dominated by bacteria and fungi, can rapidly respond to environmental changes, and the alteration of microbes is initially reflected at the individual performance (De Long et al., 2016; Nguyen et al., 2021; Wieczynski et al., 2021). When global changes occur, those microorganisms that benefit from the changes could proliferate rapidly at the cost of other microbes. Along with the individual population changes, the community would re-assemble and a shift of local microbial composition would be expected (Smith et al., 2009; Yang et al., 2019). However, the sensitivity of soil microbiota to multiple global change drivers varies to a large degree, and different environmental drivers may mutually interact to obfuscate any single effect (Rinnan et al., 2007; Sun et al., 2019). Although a growing body of evidence suggests that ecosystem processes and functions respond to multiple global changes, multi-factorial experiments that study microbial composition are largely lacking to date (Rillig et al., 2019).

Changes in soil microbial community responding to global environmental drivers can further depend on previous environmental changes, such as past land-use (Perring et al., 2016). Land-use change, like forest clearing for agriculture, can affect the soil microbial community structure and assembly through shifts in plant communities and soil management practices (Veldkamp et al., 2020; Zhou et al., 2022). To date, effects of land-use history are well-documented in temperate forest ecosystems, with dominance on community dynamics focusing on the aboveground part in particular (Perring et al., 2016). The altered plant community caused by land-use changes can directly influence the soil microbial community through release of root exudates, or indirectly influence the belowground components via alterations in quality and quantity of litter fall (Wardle et al., 2004; Feng et al., 2022). Interactions between specific soil microorganisms with their host species (i.e., mutualistic or parasitic) would also be expected to affect the belowground microbial composition (Miki, 2012; Fabianska et al., 2019). Among forest plants, herbaceous understory layer was particularly well-studied as the life-history traits of these species were closely correlated with land-use history (Verheyen et al., 2003). To better understand the mechanisms behind the soil microbial changes under different land-use legacies, it is of significance to not only focus on the responses of the understory plants or the responses of the soil microbes separately, but also quantify co-structure between them (von Rein et al., 2016).

Here we assess the microbial communities, in terms of relative abundance of identified functional groups and overall community composition, in response to four environmental change drivers, i.e., warming, N enrichment and enhancing light availability, with a distinct focus on the land-use legacies. We used phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA)-based analyses to quantify the total living biomass and especially focus on the profiles of the active part of the microbial community (Frostegard et al., 2011; Orwin et al. 2018), which could expand our knowledge on the multiple interactive effects of global change on active belowground communities. We conducted a full-factorial mesocosm experiment including all these treatments on soils from ancient temperate deciduous forests. We hypothesized that: (1) land-use history will affect the composition of the soil microbial communities, wherein

less arbuscular mycorrhizal fungi (AMF) were expected in post-agricultural forests due to the loss of forest specialists; (2) light availability and warming may induce higher soil microbial biomass, whereas N addition may not in this case as numerous European temperate forests are N saturated (De Schrijver et al., 2008); (3) the soil microbial and plant community will co-vary, as both communities are expected to respond concurrently to the applied global environmental change drivers or land-use legacies.

Materials and methods

Mesocosm setup

Study area

The mesocosms were set up in the Aelmoeseneie forest (50°58'30″ N, 3°48'16″ E, 20 m a.s.l), which is an ancient temperate deciduous forest in northern Belgium. The mean annual temperature (MAT) is 10.6 °C and the mean annual precipitation (MAP) is 768 mm (DEIMS-SDR Database) over the period November 2015-February 2016 and measured in the weather station of long-term ecological research (LTER) site. The dominant tree species are *Fagus sylvatica* L., *Quercus robur* L, *Acer pseudoplatanus* L., and *Fraxinus excelsior* L. The total throughfall subcanopy atmospheric nitrogen deposition in 2016 was 15.5 kg ha^{-1} (DEIMS-SDR Database, 2019).

Experimental design

We performed a full-factorial experiment with 64 mesocosms (2 regions× 2 pairs of forest patches × 8 treatments) to reveal the effects of global environmental changes, i.e., warming, illumination, N addition and past land-use, on the forest soil microbial community (Fig. S1).

Land-use legacies: ancient forests vs post-agricultural forests We collected soil from two regions with different soil characteristics, and contrasting past agricultural intensity within the local forests (further details in Blondeel et al., 2019). The selected forests were located in southern Sweden (SW) and northern Belgium (VL). Within each selected region, two adjacent pairs of ancient or post-agricultural broadleaf forest with a similar canopy composition were included to compare the land-use history. The ancient forest patches exist since at least 1850; while the post-agricultural forests were identified as reforestations on abandoned fields after 1950. The detailed site location and major attributes of these forests can be found in Table S1. The surface soil (15 cm depth) in each forest patch was collected, and all the collected soil samples were transferred to Aelmoeseneie forest and used to build mesocosms after sieving (5-mm mesh).

Plant community assembly: slow, intermediate and fast colonizers The plant species in the mesocosms were selected according to the recorded observations of understory communities in ancient forest or post-agricultural forests across Western Europe (Verheyen et al., 2003). The species were classified as three functional groups based on the capacity to successfully colonize into post-agricultural forests: slow colonizers and strictly forest specialists (group A), intermediate colonizers and species regularly found in post-agricultural forests but not strictly forest specialists (group B), and fast colonizing nitrophilous species (group C). The classifications and combinations of species can be found in Table S2 and S3 (more details see Blondeel et al., 2020). We used 64 mesocosms, resulting in 1280 plant individuals (64 mesocosms × 5 species × 4 individuals). We performed three rounds of weeding to remove spontaneous species during the first growing season, i.e., May, June, and September 2016.

Manipulating the environment: warming, illumination, and N addition The first treatment is experimental warming the air and soil by open-top chambers (OTCs, 75 cm wide). During the first half of sampling period (March 2017 to May 2017), the daily mean temperature of the air was significantly increased by 1.04 ± 0.47 °C ($p < 0.05$), while soil temperature only increased 0.46 °C at the surface and 0.13 °C at 5 cm depth compared with the control treatments ($p > 0.05$). During the later sampling period (June 2017 to August 2017), no significant warming effects were observed between the warming and control treatments for both air and soil (Blondeel et al., 2020). The second treatment is enhancing light availability by two 18 W fluorescent tubes, which were suspended at 75 cm above the soil surface in

each plot. In illuminated plots, photosynthetic active radiation (PAR) was significantly increased to 23.98 ± 4.40 μmol m^{-2} s^{-1} compared with the unilluminated plots (7.79 ± 0.68 μmol m^{-2} s^{-1} in 2017). The unilluminated plots only received ambient light but were equipped with dummy lamps to avoid other side effects caused by lamp installation (e.g., precipitation). The third treatment is N addition by adding 250 mL of a 2.01 g L^{-1} solution of NH_4NO_3 (50 kg N ha yr^{-1} eq.) to the N addition-treatment mesocosm, and an additional amount of 250 mL demineralized water was used to rinse the leaves to avoid the adherence of N to the plant tissues. The control plots received 500 mL of demineralized water. All the treatments (for both N addition and control) were performed four times per year at the beginning of each season. More details about the mesocosm setup can be found in Blondeel et al., 2020.

Data collection

The experiment started in April 2016 and data for the microbial analysis was collected in April 2020. From each mesocosm, five subsamples of surface soil (5 cm depth) were collected and then thoroughly mixed. To avoid contamination, the sampling spoon was disinfected with 75% ethanol between every mesocosm. The mixed soil was sieved through a 1 mm mesh and divided into two bags, as one subsample was used for microbial analysis and another for soil biogeochemical characterization.

Soil microbial community

The phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) were extracted to evaluate the soil microbial biomass and the composition of the microbial community. The subsamples for microbial analysis were stored in the freezer at -20 °C, and the PLFA and NLFA extraction were performed according to Quideau et al. (2016). PLFA/NLFA analysis mainly included three steps: extraction, lipid fractionation and lipid methylation. Methyl nonadecanoate (MeC 19:0) was used as internal standard and the concentration of each biomarker was expressed in μg g^{-1}. More details regarding the microbial experiment are provided in Yang et al., 2022a.

A total of 25 PLFA biomarkers and 5 NLFA biomarkers were detected, wherein 6 PLFA (C18:0, C18: 1ω11c, C18: 2ω9c, C18: 3ω3c, C18: 3ω6c, C20:0) and 4 NLFA (C14:0, C15:0, C16:0, C16: 1ω7c) were discarded in the following analysis because they could not easily be assigned to a particular taxonomic group or present low frequency in our dataset. The PLFA and NLFA biomarkers were classified to six functional groups. We used the sum of PLFA iC 14:0, iC 15:0,aC 15:0, iC 16:0, iC 17:0 and aC 17:0 to indicate Gram-positive bacteria (Gm+) (Farrell et al., 2013; Kaiser et al., 2015); the sum of PLFA 16:1ω7c, cy 17:0 and cy 19:0 to indicate Gram-negative bacteria (Gm-) (Mitchell et al., 2015); the sum of PLFA 10MeC16:0, 10MeC17:0 and 10MeC18:0 to indicate Actinobacteria (Act) (Xu et al., 2019); the sum of PLFA C14:0, C15:0, C16:0 and C17:0 to indicate non-specific bacteria (NSB) (Steinbeiss et al., 2009; Willers et al., 2015); and finally, the sum of PLFA 18:2ω6c and 18:1ω9c to indicate saprotrophic fungi (SF) (Zhang et al., 2014). To minimize the influence of the background amounts (from bacteria) of PLFA 16:1ω5c on the estimation of arbuscular mycorrhizal fungi (AMF) biomass in soil (Olsson, 1999; Ngosong et al., 2012), the ratio of NLFA and PLFA 16:1ω5c was used to indicate arbuscular mycorrhizal fungi. Since the NLFA/PLFA ratio was high (> 1) for most of samples in this study, we consider the PLFA 16:1ω5c to be a good proxy for AMF biomass herein (Zhang et al., 2020; Lekberg et al., 2022). The total microbial biomass was calculated by the sum of all representative biomarkers of functional groups. The relative abundances of microbial groups (i.e., Gm+, Gm-, Act, NSB, SF and AMF) were calculated as the percentage of the representative biomarkers divided by the total microbial biomass.

Edaphic properties

Soil moisture was gravimetrically determined by air-drying the soil samples at 50 °C for 48 hours in a drying oven (Reynolds, 1970). Additional subsamples used for chemical analyses were first dried at 40 °C before the further measurements. In brief, total carbon and nitrogen were quantified by combustion at 1200 °C using CNS elemental analyzer (vario Macro Cube, Elementar, Germany) (Kowalenko, 2001). Total P was measured after a complete soil digestion by using $HClO_4$ (65%), HNO_3 (70%) and H_2SO_4 (98%) in Teflon bombs for 4 h at 150 °C (Lajtha et al., 1999). Other mobile soil cations including potassium,

magnesium and aluminum were extracted by lactic acid (88%), acetic acid (99%) and ammonium acetate (25%) at pH 3.74 and then measured by atomic absorption spectrophotometry (Blondeel et al., 2019). To calculate the proportion of exchangeable base cations (BC), we convert their concentration from mg/kg to meq/kg in which the charge of the cations would be considered (Reganold and Harsh, 1985). Soil pH was measured with a glass electrode (Orion, model 920A) after shaking a soil/H_2O mixture (1:5) at 300 rpm for 5 min.

Plant community

We measured total vegetation cover (%) as the one-sided projection of vegetation in each mesocosm, which was measured by taking digital RGB photographs perpendicular to the ground surface in April 2020. The *Canopy Area* tool was used to assess the green pixels of vegetation recalculated as percentage cover (Easlon and Bloom, 2014).

Statistical analysis

All statistical analyses were carried out in R (ver.4.0.3) (Team, R.C, 2008). First, we calculated the relative abundance (%) of each microbial group by using the sum of representative PLFA biomarkers divided by the total microbial biomass. To assess how environmental changes and land-use history affected the microbial abundance as well as total microbial biomass, we used linear mixed-effects models (LMMs) to test factor variables (warming, illumination, nitrogen addition, and land-use legacy) effects on microbial communities. A total of eight response variables were tested with LMMs, i.e., relative abundance of six functional groups, ratio of Gm+ and GM- (GM+: GM-), and total microbial biomass (which was log-transformed to meet the normality assumption of the statistical tests). Then we tested the above four treatments (warming, illumination, nitrogen addition and past land-use) as fixed effects separately in our LMMs, with the "region" factor variable as a random effect. To check the effect of environmental changes and land-use history on edaphic properties, LMMs with the same fixed and random effects were built, where response variables soil K concentration and total P were log-transformed to fit normal distribution before the LMMs construction. To explain the relationship between edaphic properties and microbial abundance/ total biomass, a model including predictor variables soil total C, total P, pH, moisture, BC, C/N, and plant coverage was assessed for the response variables, i.e., relative abundance of each microbial group and total microbial biomass. To limit multicollinearity issues in the models, we performed Spearman correlation (Fig. S2) among the predictors, wherein soil total N was highly correlated with other variable (total C, $r = 0.95$) so as to discard in the following LMMs (Fig. S2). To further simplify the models, a single best model was selected based on the Akaike's Information Criterion (AIC) using the *dredge* function of the *MuMIn* package (Bartoń, 2018).

To examine the variability of the microbial community composition among environmental changes, land-use history as well as sampling regions, non-metric multidimensional scaling (NMDS) based on the Bray Curtis distance matrix were performed using *metaMDS* function in the *vegan* package (Oksanen et al., 2017). As the ordinations were strongly disturbed by the fungal biomarkers (with very low abundances), we only focused the bacterial community in this case. The goodness of fit for data calculation (NMDS) could be found in Shepard diagram (Fig. S3). To test the significance of the variation of the microbial composition among different treatments, land-use history and regions, we used analysis of similarities (ANOSIM) with function *anosim* (package *vegan*). Besides, to investigate the relationship between microbial composition and soil abiotic properties, and further screen out the most important drivers affecting the microbial composition in the soils, we performed canonical correspondence analysis (CCA). The best model was selected based on AIC by *step* function, and the collinearity among the constraining variables was checked by Variance Inflation Factor by *vif.cca* function (only variables with VIF<10 were preserved).

To assess the co-structure between the soil microbial communities and plant communities, we performed co-inertia analysis (COIA) which allows measuring the concordance between two multivariate datasets within the same object (mesocosms in this case). We first performed *Hellinger* transformation to both of the datasets and then applied COIA on a different level: i) microbial functional groups v.s. plant species, ii) PLFA biomarkers v.s. plant

species (function *coinertia* in *ade4* package) (Dray and Dufour, 2007). Besides, to better discern the co-structure between plant and different microbial communities, PLFA biomarkers were further split into bacterial biomarkers and fungal biomarkers, which were then used to conduct the COIA with the plant community, respectively (plant vs bacterial communities and plant vs fungal communities). We evaluated the strength of the coupling pairs of datasets with the RV coefficient, and this global similarity measurement could give an overview of the correlation of the dataset pairs. This coefficient value varies between 0 and 1; the closer the RV value approaches to 1, the stronger the correlation between the two datasets. We finally used Monte-Carlo test (999 random permutations) to test the significance of the co-structure between the datasets.

Results

Soil microbial abundance and associated environmental properties

In general, the total microbial biomass in soils ranged from 2.94 to 37.50 μg g^{-1}. Except for illumination (marginally significant), no significant differences were found among treatments. Most (~80%) of the obtained biomarkers (excluding undetermined biomarkers) were indicative for bacteria, with Gram-positive bacteria having the highest relative abundance (33.71% on average). Several fungal biomarkers were also detected in the soil samples, but the relative abundance (6.73% on average) was comparably lower than those of bacteria (21.64% on average). When we compared the relative abundances of these functional groups among four different treatments, we found that past land-use exerted a significant impact on the soil microbial abundance, in which AMF and Actinobacteria showed a higher relative abundance in ancient forests (+4.33 ± 1.79 and 1.09± 0.46, respectively), while non-specific bacteria showed a higher relative abundance in post-agricultural forests (+3.63 ± 0.91, Fig. 1 and Table 1). The illumination had a marginally significant effect and led to a higher microbial biomass (+3.33 ± 1.53, $p = 0.06$). Saprotrophic fungi had a higher relative abundance (+3.51 ± 1.74, $p = 0.08$), while Gram-positive bacteria had lower abundance (-3.89 ± 1.93, $p = 0.08$) in response to light addition. In contrast, the soil microbial abundance was not significantly affected by the warming nor nitrogen addition ($p > 0.1$).

Past land use showed also significant effects on the soil abiotic properties and plant coverage. As shown in Table S4, post-agricultural forests had higher soil pH (+0.37 ± 0.07), total P (+277.92 ± 34.60) as well as plant coverage (+18.59 ± 5.13) than ancient forests ($p < 0.001$), while ancient forests had higher soil moisture (+9.98 % ± 1.87, snapshot data during our sampling), total C (+0.57 % ± 0.24) and C/N ratio (+1.14 ± 0.34) instead ($p < 0.01$). Then we used the simplified LMMs to reveal the correlations of these properties to the relative abundance of microbial groups and total microbial biomass and found significant correlations of microbial abundance and soil C and C/N ratio (Table 2). The relative abundance of Gram-positive bacteria was positively correlated with soil C and negatively correlated with C/N ratio, while Actinobacteria were only positively correlated with soil C.

Soil microbial composition and associated environmental properties

NMDS ordination and ANOSIM revealed significantly different community composition in soil bacteria between both past land-use classes, but no significant effects were found for other experimental treatments (warming, illumination and nitrogen addition) (ANOSIM, $p > 0.05$; Fig. 2). CCA was performed to investigate which environmental properties contributed to explain the variation in soil microbial community composition. As shown in Fig. 3, the first two axes explained 14.47% of the variance in bacterial community composition, and the variation was significantly related to soil pH and C/N ratio ($p = 0.04$ and $p = 0.01$, respectively & Table S5).

Co-structure between the soil microbial and the plant community

There was no significant co-structure between the soil microbial groups or PLFA biomarkers (for both bacterial and fungal biomarkers) and plant communities across all plots (Table 3). However, when we split the dataset into different past land-use classes, we found a significant co-structure in the ancient, but not in the post-agricultural soils (Table 3). In order to visualize

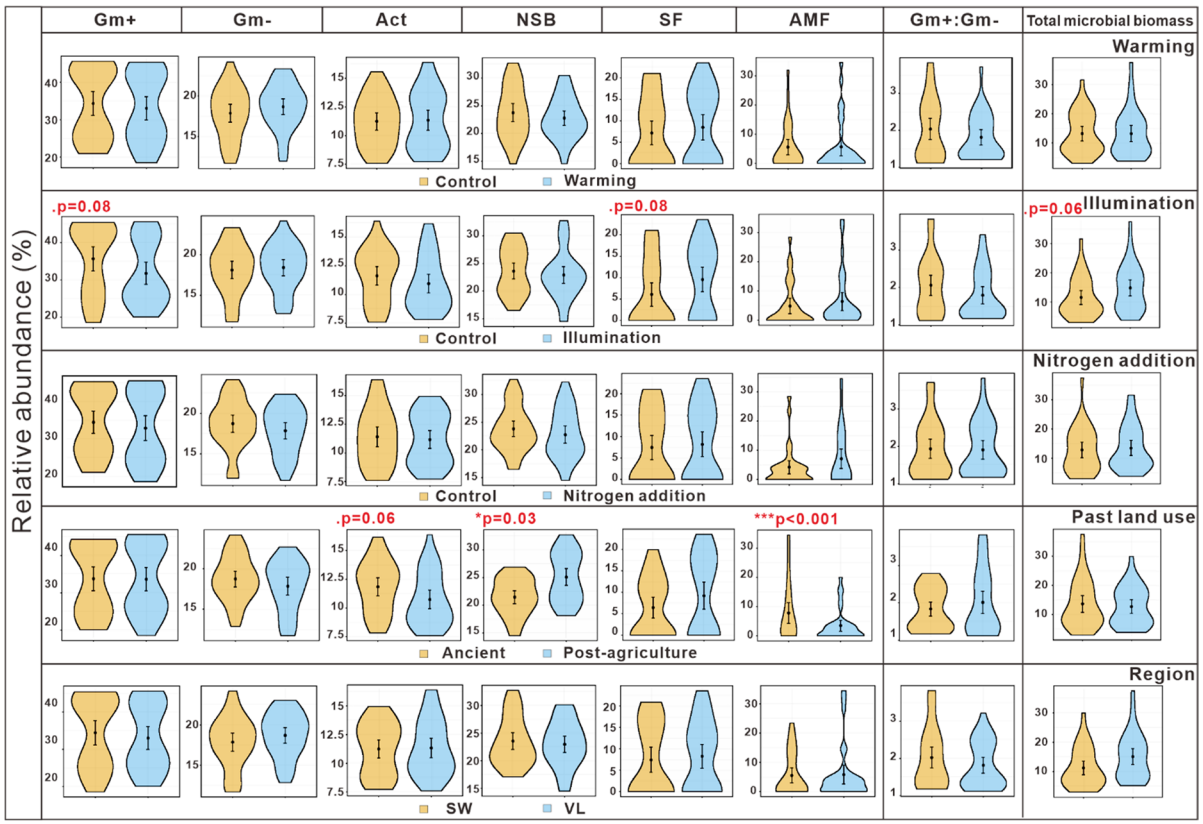

Fig. 1 Relative abundances (%) of microbial groups, ratio of Gram-positive and Gram-negative bacteria, and total microbial biomass (μg g^{-1}) of the experimental treatments. Gm+, Gram-positive bacteria; Gm-, Gram-negative bacteria; Act, Actinobacteria; NSB, non-specific bacteria; SF, saprotrophic fungi; AMF, arbuscular mycorrhizal fungi; SW, southern Sweden; VL, northern Belgium

the relative abundance of plant community that affected microbial community, projections of the first two axes of COIA were presented for all 6 microbial groups and 19 plant species (Fig. 4a). The first two axes explained 97% of the total co-variation between the soil microbial and plant communities. A shorter arrow in ancient forests (1.22 ± 0.05, Mean ± S.E.) than post-agricultural forests (1.34 ± 0.07) indicated a stronger relationship between plant and microbial communities in the former. Besides, the positions of each indicator in panels (b) and (c) represent the importance and the direction of their contribution to the distribution of sites in the COIA shown in panel (a). In that case, microbial functional groups (in panel b) and plant species (in panel c) projecting in the same direction from the origin have a strong association. Based on our results, CIOA showed that bacterial groups (Gram-negative bacteria, Actinobacteria, Gram-positive bacteria and non-specific bacteria) were strongly related to plant species like *Quercus robur*, *Geranium robertianum*, *Rubus fruticosus*, and *Fraxinus excelsior* L. In contrast, saprotrophic fungi were associated with *Polygonatum multiflorum* L. and *Carex sylvatica* Huds., and AMF were associated with *Hyacinthoides non-scripta* and *Poa trivialis* (Fig. 4b & c).

Discussion

Land-use history: keystone driver in shaping microbial community structure

We found that land-use history was the prominent driver for shaping microbial community structure, rather than the *in situ* applied environmental changes.

Table 1 Effects of warming, illumination, nitrogen addition and land-use history on the relative abundance of microbial groups and total microbial biomass based on linear mixed-effect models. Statistically significant Chi-square values are highlighted in bold

Relative abundance	Warming				Illumination				Nitrogen addition				Land-use history			
	Chi-square value	p value	Conditional R^2 (%)	Marginal R^2 (%)	Chi-square value	p value	Conditional R^2 (%)	Marginal R^2 (%)	Chi-square value	p value	Conditional R^2 (%)	Marginal R^2 (%)	Chi-square value	p value	Conditional R^2 (%)	Marginal R^2 (%)
Gram-positive bacteria	0.32	0.57	0.5	0.5	**3.02 .**	**0.08**	4.57	4.57	0.47	0.49	0.75	0.75	<0.01	0.965	0.004	0.004
Gram-negative bacteria	1.11	0.29	2.46	1.71	0.15	0.70	0.92	0.23	1.35	0.25	2.83	2.08	1.41	0.23	2.93	2.18
Actinobacteria	0.03	0.85	0.05	0.05	1.38	0.24	2.14	2.14	0.17	0.68	0.27	0.27	**3.63 .**	**0.06**	5.45	5.45
Non-specific bacteria	0.88	0.35	1.37	1.37	0.47	0.49	0.75	0.75	0.91	0.34	1.43	1.43	**13.88 *****	**<0.001**	18.05	18.05
Saprotrophic fungi	0.39	0.53	0.62	0.62	**3.13 .**	**0.08**	4.72	4.72	0.14	0.71	0.22	0.22	1.96	0.1617	3.01	3.01
Arbuscular mycorrhizal fungi	<0.01	0.95	0.01	0.01	0.54	0.46	0.86	0.86	2.12	0.15	3.26	3.26	**4.75 ***	**0.03**	7.01	7.01
Gm+: Gm-	1.53	0.22	3.32	2.34	2.22	0.14	4.38	3.37	0.03	0.87	0.94	0.04	0.96	0.33	2.44	1.49
Total microbial biomass §	0.02	0.88	12.56	0.03	**3.66 .**	**0.06**	17.47	4.8	0.35	0.55	13.02	0.49	0.05	0.82	12.6	0.07

Asterisks indicate significances as: . p<0.01;* p < 0.05; *** p<0.001. § means this variable was log-transformed.

Table 2 Effects of soil total C, C/N ratio, total P, pH, and base cations on the relative abundance of each microbial group and total microbial biomass based on linear mixed effect models. Chi-square values of each specific microbes and total microbial biomass were reported. Statistically significant Chi-square values were highlighted in bold

	Soil C	Soil C/N	Soil P §	Soil pH	Base Cations	Conditional R^2 (%)	Marginal R^2 (%)
Gram-positive bacteria	**19.6*****↑	**18.2*****↓	1.1	0.5	0.1	28.7	28.7
Gram-negative bacteria				0.2	<0.1	11.8	0.4
Actinobacteria	**4.6***↑				<0.1	7.2	11.4
Non-specific bacteria			0.3	<0.1	<0.1	17.1	0.6
Saprotrophc fungi	1.7	2.6	<0.1	<0.1	0.3	4.5	4.5
Arbuscular mycorrhizal fungi	0.3		2.1	0.5	0.1	6.0	6.0
Total microbial biomass§	**7.0****↑	**8.3****↓	0.7	0.4	<0.1	14.0	14.0

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Symbol '§' followed by P indicated that data were log-transformed during preprocessing.

The land-use legacy significantly changed the microbial abundance and community composition (Fig. 1 & 2d). We showed a higher abundance of arbuscular mycorrhizal fungi (AMF) and Actinobacteria in ancient forests, which confirms our first hypothesis. In ancient forests, functional traits of understory plants show adaptation to low-light conditions (Valladares & Niinemets, 2008). These understory species often emerge in early spring and benefit from nutrients stored in belowground tissues (Verheyen et al. 2003). When the photosynthesis of plants decreased due to the low light intensity or shading, AMF communities could aid plants with nutrient and water acquisition with the nutrient uptake via plant root system, hence highlighting their dominant ecological niche in ancient forests (Xu et al., 2018; Kaur and Suseela, 2020; Wen et al., 2022). Ancient forests soils are also expected to have higher carbon accumulation in soils compared to those in post-agricultural forests (Fanin and Bertrand, 2016). Actinobacteria are one of the most representative copiotrophic bacterial members, i.e., preferring to colonize soils rich in organic matter and characterized with a relatively rapid growth rate (Selene Gomez-Acata et al., 2016; Yao et al., 2017). Therefore, the abundant C accumulation in ancient forest soils facilitates the enrichment of Actinobacteria herein (see Table S4). Furthermore, previous literature has shown that α-diversity of AMF and several bacterial species will decrease as the land use changes (Berkelmann et al., 2020; Ji et al., 2022), which could partially support our findings (increase of AMF and Actinobacteria abundance in ancient forest soils). The colonization of soil microbial communities is mainly dependent on the organic layers and nutrient availability in forests (Wilson and Puri, 2001; Zhou et al., 2006), and this would reasonably result in the microbial species in ancient forests generally having a longer time for colonization (Boeraeve et al., 2018).

The beta-diversity analysis (NMDS ordination), which displayed the overall community composition of soil bacteria, showed clear changes between both land-use legacies (Fig. 2d). The differences between land-use types may be in part be explained by differences in soil chemical properties, as soil pH was one of the most important drivers affecting the microbial composition in the soils (Fig. 3 and Table S5). Due to past fertilizing, liming and manuring practices, post-agricultural deciduous forests with an intensive agricultural history generally present a higher pH compared to ancient forests (Verheyen et al., 1999; von Oheimb et al., 2008; Orczewska, 2009; Blondeel et al., 2019). On the one hand, pH can affect the microbial structure directly, where the microbial community changes from acidophilic microbes to neutrophilic microbes during the agricultural period (Chen et al., 2021; Sridhar et al., 2022). On the other hand, these edaphic properties might have an effect on aboveground plant species composition first and then exert a legacy effect on the underground community. For example, the changes of litter composition and humus type caused by altered plant community composition can result in indirect effects on the microbial community structure in different land-use legacies (Santonja et al., 2017; Bayranvand et al., 2021). However, the response strength of the understory traits to the edaphic property

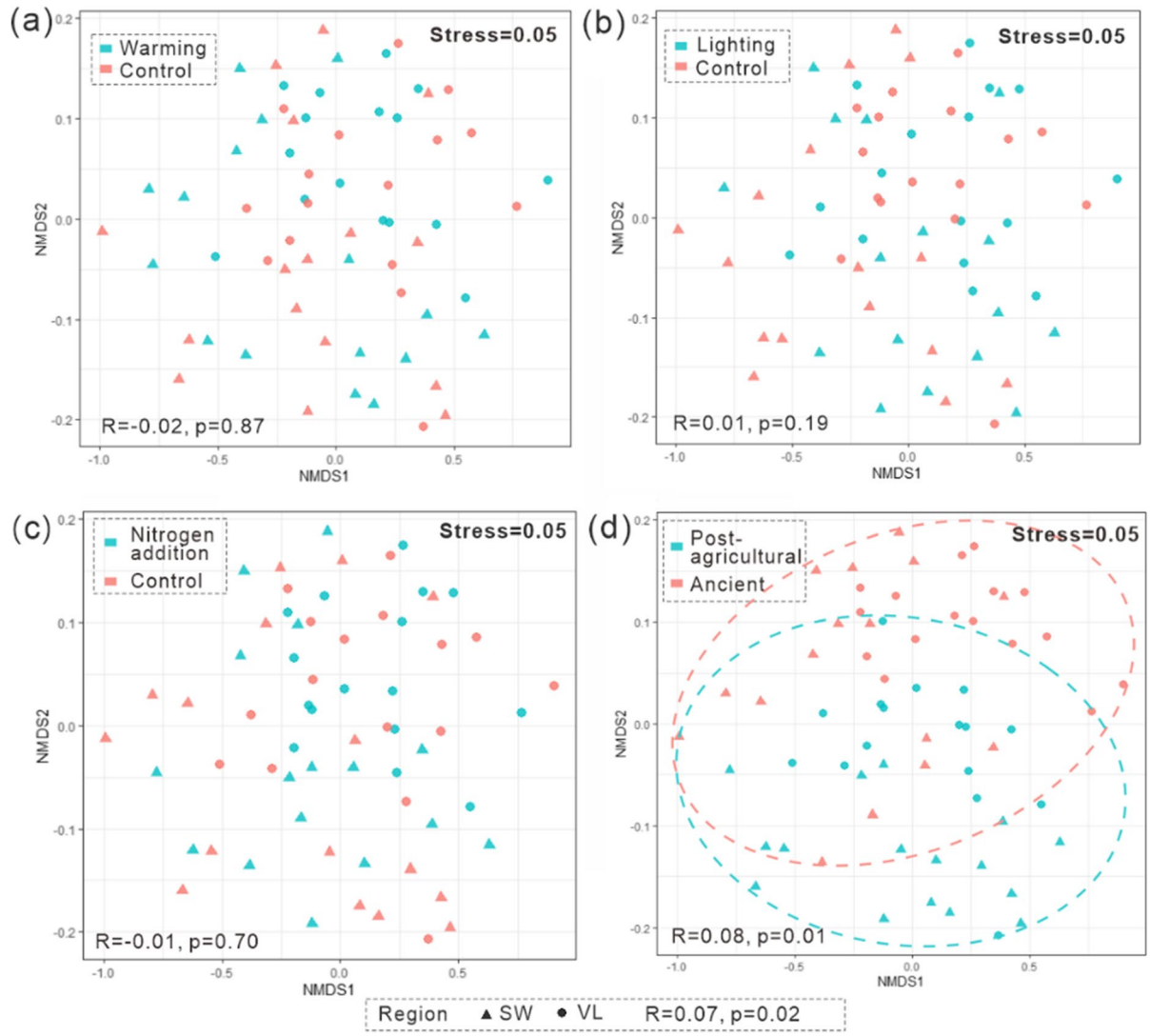

Fig. 2 Non-metric multidimensional scaling (NMDS) results of the bacterial composition based on PLFA biomarkers. Variations of the bacterial composition between different experimental treatments were calculated based on the Bray-Curtis distance matrix. Dissimilarity values based on analysis of similarities (ANOSIM) are also shown in plots. SW, southern Sweden; VL, northern Belgium

variations can be further dependent on the background flora (local plant species pool) and detailed land-use practice (Falkengren-Grerup et al., 2006; Barlow et al., 2020). The soil microbial structuring mechanisms in forests with contrasting land-use legacies, and their linkages with understory species and edaphic properties, require further experimental tests and analysis.

Environmental change: additional illumination increases total microbial biomass and affect part of their abundance

Light availability emerged to increase the soil microbial biomass and also change the relative abundances of several microbial groups in our experiment. This result supports our second hypothesis that when

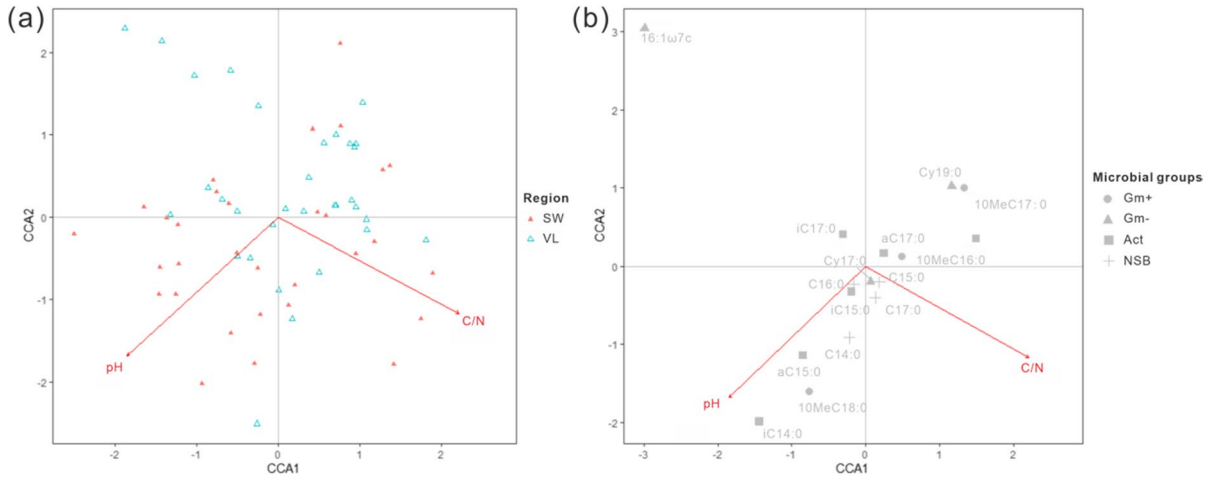

Fig. 3 The relationship between bacterial community composition and abiotic properties (soil characteristics) based on canonical correspondence analysis (CCA). The first axis accounted for 10.58% of the variability and the second axis accounted for 3.89%. The significance levels of CCA1 and CCA2 were p = 0.02 and p = 0.08, respectively (permutation test). (a) The distribution of samples collected in different regions. Different colors in this panel indicated the soil sources. SW, southern Sweden; VL, northern Belgium. (b) The distribution of single PLFA biomarkers. Different symbols in this panel indicated the classified microbial groups. Gm+, Gram-positive bacteria; Gm-, Gram-negative bacteria; Act, Actinobacteria; NSB, non-specific bacteria

Table 3 The co-structures between the soil microbial and plant communities in all plots and subset plots according to land-use legacies

Soil microbial communities	RV coefficient		
	All plots	Ancient forests	Post-agricultural forests
Microbial groups (6)	0.08	**0.21***	0.09
PLFA biomarkers (19)	0.09	0.13	0.09
Bacterial biomarkers (16)	0.09	0.13	0.09
Fungal biomarkers (3)	0.08	0.12	0.08

PLFA, phospholipid fatty acids. * p<0.05 (Monte Carlo test). RV, correlation (R) of vectors.

illumination is applied, the aboveground plant productivity increases and more litter inputs and root exudates are expected to flow to the soil food web, which lead to a higher soil microbial biomass and activity. Indeed, additional illumination can also regulate the root exudates and rhizodeposition to enhance the microbial biomass or shift soil microbial composition (Hristozkova et al., 2017; Xi et al., 2019). For example, Balasooriya et al. (2014) used ^{13}C pulse-labeling to assess the role of soil microbes in the cycling of rhizodeposit-C. They found that fungi enable to form a rapid channel of rhizodeposit-C into the soil microbes, while Actinomycetes and gram-positive bacteria presented a delayed utilization. Above conclusions support the results in this study, i.e., saprotrophic fungi showed a higher relative abundance under the illuminating treatment while Gram-positive bacteria showed a lower relative abundance instead (Fig. 1). These results may indicate the increasing root exudates caused by illumination can accelerate the enrichment of saprotrophic fungi rather than gram-positive bacteria, which may prefer other C sources instead of rhizodeposition (Bird et al., 2011; Balasooriya et al., 2014). However, no significant enrichment of AMF in soils was observed when additional illumination was applied. This is to say, even with the additional illumination, the plant investment in an already established mycorrhizal network could impose a lower carbon cost (Teste et al., 2009; Unger et al., 2021), which may inhibit the response of AMF to the additional light availability. Furthermore, the light effects on AMF were equivocal in previous

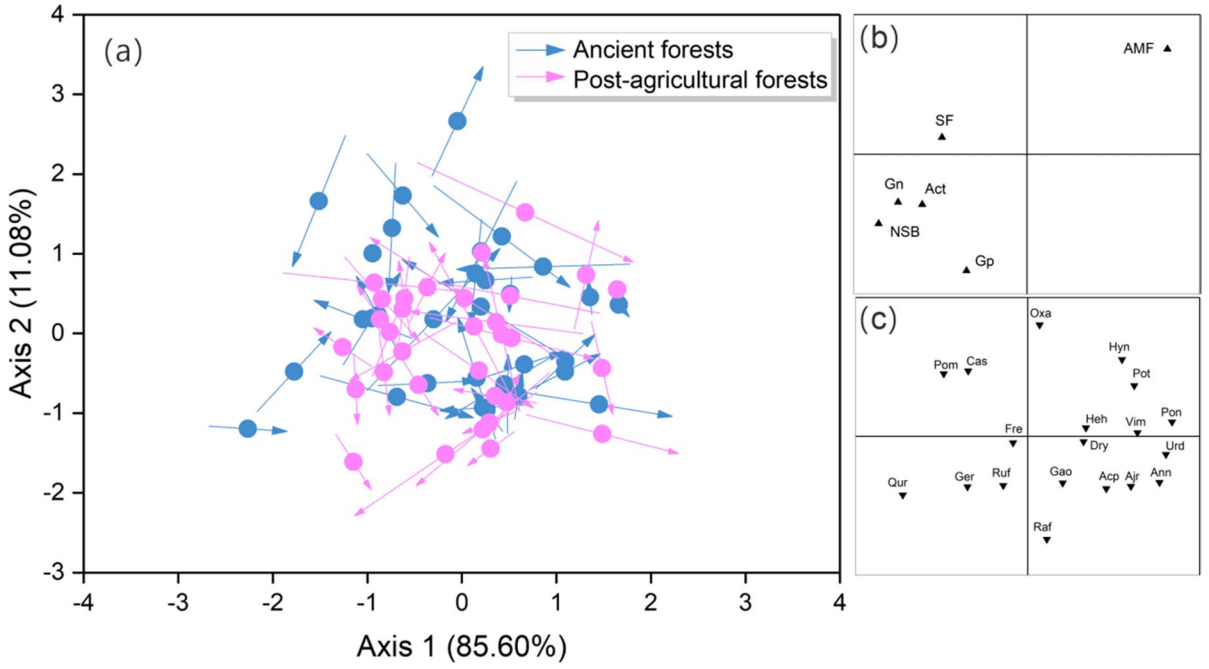

Fig. 4 The co-inertia analysis of the soil microbial and plant community composition across all plots. (**a**) The positions of each plot on the first two axes of the COIA is conditional on the plant (arrow tails) or microbial (arrow heads) communities. The colours of the arrows show the site groups according to the past land-use. The percentages of variance explained by axes are shown on each axis. (**b**) Projections of the microbial groups and (**c**) projection of the plant species. The abbreviations of the bacterial phyla in (b): Gm+, Gram-positive bacteria; Gm-, Gram-negative bacteria; Act, Actinobacteria; NSB, non-specific bacteria; SF, saprotrophic fungi; AMF, arbuscular mycorrhizal fungi. The abbreviations of the plant species in (c): Acp, *Acer pseudoplatanus* L.; Ajr, *Ajuga reptans* L.; Ann, *Anemone nemorosa* L.; Cas, *Carex sylvatica* Huds.; Dry, *Dryopteris*; Fre, *Fraxinus excelsior* L.; Gao, *Galium odoratum* L.; Ger, *Geranium robertianum* L.; Heh, *Hedera helix* L.; Hyn, *Hyacinthoides non-scripta* L.; Oxa, *Oxalis acetosella* L.; Pom, *Polygonatum multiflorum* L.; Pon, *Poa nemoralis* L.; Pot, *Poa trivialis* L.; Qur, *Quercus robur* L.; Raf, *Ranunculus ficaria*; Ruf, *Rubus fruticosus* agg.; Urd, *Urtica dioica* L.; Vim, *Vinca minor* L. We refer to the text for further info on the interpretation of the figure

literature, and more detailed data on the soil nutrient availability, AMF taxa and ecosystem types should be explicitly considered in the future (Liu et al., 2015; Ballhorn et al., 2016; Ficano et al., 2021; Yang et al., 2022b).

Plant communities: co-varied with soil microbial community, but only in ancient forest

The significant relationship between the soil microbial and plant communities in ancient forest soils supports the idea that land-use history affects the soil microorganisms through a legacy effect on the understory plants. First, we found that the fast-colonizing, generalist plant species including *Poa trivialis* were positively correlated with AMF, whereas slow-colonizing plant species such as *Polygonatum multiflorum* and *Carex sylvatica* were positively correlated with saprotrophic fungi based on the COIA analyses (Fig. 4b & c). These findings corroborate that in an acid nutrient-poor ancient forest soil, communities with slow-colonizing species are expected to be dominant, whereas in less acid and nutrient-rich post-agricultural forest, the plant community is dominated by fast-colonizing species (Verheyen et al., 2003; Wardle et al., 2004). Second, the significant linkages of plant and soil microbial communities in ancient forest soils could be the result of similar compositional responses of aboveground and belowground communities responding to the same soil characteristics. Additionally, the indirect effects of land-use history on belowground community compositions due to changes that occur in plant functional traits can also contribute, as we also found a significant difference

of total vegetation cover and cover-weighed community weighted means of SLA (specific leaf area) between ancient and post-agricultural forest in the second growing season of the experiment (March to September in 2017, see Blondeel et al., 2019 and Marri, 2020).

However, we found no significant co-structure between plant and soil microbial community in post-agricultural forest soils. It is known that plant-microbe community dynamics are complex and characterized by instability, therefore different land-use legacies could evoke a series of plant-microbe community shift (Bardgett et al., 2005; Kardol et al., 2006). For example, due to past fertilizing and manuring practice, post-agricultural forest soils represent chronic nutrient input, as such it would facilitate the growth of those plant/microbes beneficial from high nutrient availability (Holt, 2008; Veresoglou et al., 2011) and eventually distort the plant/microbial linkages compared to those in ancient forests. Besides, the different co-structure between plant and microbial community in this study would also be linked to the divergence of plant communities between the two past land-use types. For ancient forests, we see the dominance of forest specialists and other species have been filtered out. By contrast, a wide array of planted species (mixture of fast- and slow colonizing plants) were present on post-agricultural soils. Furthermore, whether the strong co-structured relationship between plant and soil microbes in ancient forest soils may act as a buffer, such as weakening understory control over other environmental changes (i.e., against extreme droughts), should be tested in future experiments. In this respect, it is essential to focus on plant-microbial relationship when studying the response of soil microbial community under different land-use legacies, certainly when interested in mechanisms of community response to environmental changes.

Conclusion

In our full-factorial mesocosm experiments with three global change drivers and past land-use included, we found that land-use legacy emerged as a critical driver for soil microbial community. In ancient forests, there was a higher abundance of AMF and Actinobacteria, a differential composition of soil bacterial communities, and the composition of the soil microbial and plant communities was also co-structured. Warming and additional N did not affect the soil microbes, while illumination did. This treatment resulted in an increased soil microbial biomass, a higher abundance of SF and a lower abundance of Gram-positive bacteria. Our results shed light on the importance of past land-use for belowground biota and the linkages with aboveground vegetation. A logical next step will be to test whether the strong linkages with plant communities in ancient forest soils may contribute to the high ecosystem resilience.

Acknowledgement This work was supported by the European Research Council via ERC Consolidator Grant PASTFORWARD (Number 614839), ERC Starting Grant FORMICA (Number 757833), the FWO Scientific research network FLEUR, China Postdoctoral Science Foundation (Number 2023M733462), and the scholarship provided by the University of Chinese Academy of Sciences (UCAS). We thank Luc Willems and Greet De bruyn during the chemical analyses, as well as Samuel Bodé and Katja Van Nieuland for providing help during the PLFA experiment.

Author contributions J.Y., H.B., P.D.F., and K.V. conceived the ideas and designed methodology; all authors collected data; J.Y. performed statistical analyses; J.Y., with contributions from H.B., P.D.F., and K.V. wrote the paper; all authors discussed the results and commented on the manuscript drafts.

Data availability Data related to this manuscript are available on Figshare: https://doi.org/10.6084/m9.figshare.21788738.v1.

Declaration

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.

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