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Microbial autotrophy explains large-scale soil CO_2 fixation

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Abstract

Microbial communities play critical roles in fixing carbon from the atmosphere and fixing it in the soils. However, the large-scale variations and drivers of these microbial communities remain poorly understood. Here, we conducted a large-scale survey across China and found that soil autotrophic organisms are critical for explaining CO_2 fluxes from the atmosphere to soils. In particular, we showed that large-scale variations in CO_2 fixation rates are highly correlated to those in autotrophic bacteria and phototrophic protists. Paddy soils, supporting a larger proportion of obligate bacterial and protist autotrophs, display four-fold of CO_2 fixation rates over upland and forest soils. Precipitation and pH, together with key ecological clusters of autotrophic microbes, also played important roles in controlling CO_2 fixation. Our work provides a novel quantification on the contribution of terrestrial autotrophic microbes to soil CO_2 fixation processes at a large scale, with implications for global carbon regulation under climate change.

KEYWORDS
autotrophic bacteria, biogeographic pattern, CO_2 fixation rate, phototrophic protists, soil carbon cycling

1 | INTRODUCTION

Soil organic carbon (SOC) represents the largest terrestrial carbon (C) reservoir and is vital to soil fertility, climate change and ecosystem services (Carvalhais et al., 2014; Davidson & Janssens, 2006; Reichstein et al., 2013). Microbial communities are critical drivers of soil carbon cycling. However, most studies have focused on investigating decomposition and mineralization processes driven by heterotrophic organisms (Bond-Lamberty et al., 2018; Jansson & Hofmockel, 2020), much less is known about the contribution of autotrophic microbes in explaining C fixation. Previous studies on C fixation and photosynthesis mainly focused on vascular and mosses in terrestrial ecosystems, yet there is still important uncertainty about the capacity of microbes to explain C fixation across large

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environmental gradients, and a large-scale quantification of the contribution of soil microbes for explaining carbon fixation patterns is largely lacking. Even so, this knowledge is urgently needed to predict changes in soil C stocks under global change.

The crucial roles of autotrophic microbes in CO_2 fixation have been well studied in aquatic systems but is largely unknown in terrestrial ecosystems. To the best of our knowledge, autotrophic microorganisms have six pathways capable of fixing atmospheric CO_2, the Calvin–Benson–Bassham (CBB) cycle being the most ubiquitous and dominant pathway in soils across the globe (Saini et al., 2011; Xiao et al., 2021). This path is performed by multiple bacteria and protist organisms. Carbon fixed by plants and autotrophic microbes undergoes different turnover processes in soils. Plant inputs are estimated to explain 20%–30% of soil carbon which is the result of litter decomposition and root residues and exudates (Liu et al., 2019). The majority of these plant input organic carbon (OC) is mineralized and lost through the release of CO_2, with only a small proportion (<5%) being long-term sequestrated into soil orgainc matter (Hutsch et al., 2002; Liu et al., 2019). Inputs of fresh OC to soil can stimulate microbial activities and biomass turnover, leading to an accelerated decomposition of the old soil organic matter (priming effects) (Bastida et al., 2019; Schmidt et al., 2011). Carbon fixed by autotrophic microbes is expected to enter into the stable SOC pools in forms of microaggregate-associated C and humin fractions (Spohn et al., 2020; Xiao et al., 2021). Microbially mediated autotrophic CO_2 fixation processes actively contribute to soil C sequestration, yet a quantification on the relative importance of autotrophic microbes for explaining C fixation is still lacking. We posit that the incorporation of microbially mediated autotrophic CO_2 fixation processes into the conceptual framework of soil carbon cycling can be fundamental to improve accurate assessments of soil C cycling and soil C sequestration.

Forest ecosystems, which occupy about one-third of the total world land area, serve as the largest and most important soil carbon pool (Akinyede et al., 2020). The intensive cultivation of both paddy and upland soils attributable to the food and fiber production may have profound consequences on the C emission, showing prominent potential in the regulation of C preservation. Agricultural soils tend to differ from natural ecosystems in soil properties and ecological processes due to human activities and managements, resulting in distinct microbes-driven carbon cycling (Jiao et al., 2019). In particular, rice paddy cultivation under long-term flooding conditions strongly alters the soil environmental factors and microbial attributes, making up distinct ecosystem processes from the upland soils (Chen et al., 2021; Liu et al., 2019). Therefore, to accurately predict soil cycling, we require a mechanistic understanding of C fixation under different ecosystems.

Here, we aimed to reduce important uncertainties in the contribution of soil microbes in explaining C fixation across large environmental gradients. In particular, we aimed: (1) to assess the spatial distributions of autotrophic bacteria and phototrophic protists and their contributions to CO_2 fixation rates at the large scale, (2) to evaluate the potential of microbial autotrophic CO_2 fixation across different terrestrial ecosystems, and (3) to identify the key ecological predictors of CO_2 fixation in soils. To achieve these goals, we determined the CO_2 fixation potential using a soil incubation of 15-day with $^{13}CO_2$ labeling in 159 soils across three terrestrial ecosystems (forest, upland and paddy) along the latitudinal gradient from N18° to N48° with a distance of over 3680 km throughout eastern China. As ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the fundamental enzyme regulating the CBB cycle, the *cbbL* gene, encoding a large subunit of RubisCO form I, was used as the molecular marker for CO_2-fixing autotrophic bacteria (Selesi et al., 2005; Xiao et al., 2014; Yuan et al., 2012). The 18S rDNA-derived phototrophic protistan communities were analyzed considering the prevalence of protist phototrophs and their contribution to CO_2 fixation. The relative importance of climate, soil and autotrophic microbial properties in shaping the spatial patterns of soil CO_2 fixation potential was further quantified. Our results indicate that microbial CO_2 fixation occurs widely and largely explains soil CO_2 fixation patterns in natural and agricultural ecosystems, and highlight the outstanding contribution of paddy soils.

2 | MATERIALS AND METHODS

2.1 | Soil sampling and data collection

A total of 159 soil samples were collected from 19 sites along latitudinal gradients throughout eastern China, including 51 forest, 54 upland, and 54 paddy soils (Figure S1). To cover broad environmental gradients, the sampling regions were selected ranging from 18.69° N to 47.45° N with a total distance of 3689 km. Soil sampling was conducted at a depth of 0–15 cm with a mixture of five randomly collected cores for each sample. At each site, the locations of the three ecosystems were adjacent and less than 10 km apart. The upland and paddy fields were planted with the main crop, and the forest areas were natural with little or no interference from human activities. The soil samples were immediately transported to the laboratory in ice-cooled containers. After removing plant residues and stones, the soils were sieved (2 mm) and then separated into two subsamples. One subsample was stored at −80°C for DNA extraction, and the other was stored at 4°C for the CO_2-fixing assay and for measuring edaphic variables.

Soil pH was measured by a pH meter (1:2.5 w/v soil to water ratio). Soil total carbon (TC), total nitrogen (TN) and $\delta^{13}C$ values (delta ^{13}C) were determined with an elementary analyzer-stable isotope ratio mass spectrometer (EI-IRMS; Elementar Vario PYRO cube and Isoprime100). The content of SOC was measured by the $K_2Cr_2O_7$–H_2SO_4 oxidation method. Soil dissolved organic carbon was extracted with 0.05 M K_2SO_4 and assessed with a total OC analyzer (Elementar Vario TOC cube). Microbial biomass carbon was measured using the chloroform-extraction method. Soil nitrate (NO_3^-) and ammonium (NH_4^+) were extracted with 2 M KCl and assessed by automated segmented flow analysis (Alliance). The available phosphorus (AP) was extracted with 0.5 M $NaHCO_3$

and measured by the molybdenum blue method. Climatic factors, including mean annual temperature (MAT) and mean annual precipitation (MAP), were obtained from the WorldClim database (https://www.world clim.org).

2.2 | Measurement of CO_2 fixation potential

To examine the atmospheric CO_2 fixation capacity, 10 g fresh soil (2 mm, air-dried equivalent) was incubated in 100 ml sealed glass jars with the addition of 5% (v/v) $^{13}CO_2$ (Liao et al., 2020; Long et al., 2015). Prior to the experiment, the moisture of upland and forest soils was adjusted to 40% water holding capacity (WHC), and paddy soils were flooded (1 cm water layer) during the incubation. The soils were preincubated for 2 days at 25°C in the dark. Then, the glass was opened and flushed with fresh air. After injection of $^{13}CO_2$ (99 atom%), soils were incubated at 25°C under alternating 12 h light/12 h dark cycles to simulate the natural environment. Gas changes and $^{13}CO_2$ injection were conducted periodically every 5 days. Soil samples were harvested after 15 days of incubation and treated with 2 M HCl for 1 day to remove inorganic carbon (Wu et al., 2014; Zhao et al., 2018). The treated soils were freeze-dried and sieved (0.147 mm). The $\delta^{13}C$ analysis was conducted using an EA-IRMS (Elementar Vario PYRO cube and Isoprime100), and the stable isotope abundance (^{13}C atom%) of the original and labeled soils was calculated based on the international standards Pee Dee Belemnite. The soil $^{13}CO_2$ fixation rate was quantified according to the increases in ^{13}C atom% of the soil after incubation.

2.3 | Illumina sequencing of the *cbbL* and 18S rRNA genes

Soil DNA was extracted using a MoBio PowerSoil kit (MOBIO) and determined with a NanoDrop 2000 (Thermo Fisher Scientific). Soil *cbbL*-containing autotrophic bacterial and phototrophic protist communities were profiled by high-throughput sequencing of the *cbbL* gene and the 18S rRNA gene V4 region, respectively. PCR amplification was conducted using the primer sets k2f/v2f for *cbbL*-containing autotrophic bacteria (Tolli & King, 2005; Yuan et al., 2012) and TAReuk454FWD1/TAReukREV3 for protists (Guo, Xiong, et al., 2021; Zhao et al., 2019). The amplicons were purified and quantified and then submitted to an Illumina MiSeq PE300 platform for sequencing. After removing the adapter and primer sequences, paired-end reads were first merged using FLASH and denoised using the DADA2 pipeline in QIIME2 (Callahan et al., 2016). Amplicon sequence variants (ASVs) were generated and assigned for taxonomy by blasting against the NCBI nonredundant database for the autotrophic bacteria and against the Protist Ribosomal Reference database (PR2 v_4.5) for protists. We excluded ASVs that were absent in less than two samples. Protist ASVs were extracted from the eukaryote ASV table by removing ASVs belonging to Rhodophyta, Streptophyta, Metazoa, Fungi, Opisthokonta_X and unclassified taxa. Protistan functional groups were manually classified according to the feeding modes. The ASVs assigned to phototrophs were selected and rarefied for subsequent analysis. Overall, a total of 15,811 ASVs of *cbbL*-containing autotrophic bacteria and 1549 ASVs of phototrophic protists were detected in this study.

2.4 | Quantitative PCR assay

The primers K2f/V2r (Tolli & King, 2005) and F-EUK528 and R-CHL002 (Zhao et al., 2020) were used to evaluate the absolute abundance of the *cbbL*-containing bacteria and Chlorophyta, respectively. Quantitative PCR (qPCR) was performed in a 20 μl volume system containing 10 ng of template, 0.8 μl of each primer at 10 μM, and 10 μl of SYBR Green Premix (Takara) on an ABI7500 FAST system. The qPCR program was 95°C for 10 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 61°C for 30 s (*cbbL*-containing bacteria) and at 60°C for 30 s (Chlorophyta), and elongation at 72°C for 30 s. All real-time PCR standard curves were generated from a 10-fold serial dilution of the positive plasmid DNA.

2.5 | Statistical analysis

The α-diversity indices (Chao 1) were examined using the 'vegan' package in R v.4.0.2. One-way analysis of variance was conducted to explore the significance of ecosystem types on CO_2 fixation rates, soil properties, microbial abundances and diversity indices. Nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis distance was carried out to profile the differences in the community structure of autotrophic bacteria and phototrophic protists across different ecosystems. Permutational multivariate analysis of variance (PERMANOVA) was used to explore the significant differences between ecosystems. Indicator species analysis was employed to obtain the individual autotrophic bacterial and phototrophic protistan indicator in upland, paddy, and forest soils with the 'indicspecies' package, and the results were visualized with a bipartite network (De Caceres et al., 2010; Hartman et al., 2018).

The biogeographic patterns of microbial similarity (1—Bray–Curtis dissimilarity) were estimated using the linear regression model. Mantel analysis was deployed to determine the impacts of climatic and edaphic variables on the microbial communities. Boosted regression trees (BRTs) was used to partition the independent effects of autotrophic bacteria, phototrophs, climatic and soil variables on CO_2 fixation rates in different ecosystems (Guo, Deng, et al., 2021). Random forest analysis was leveraged to predict the importance of autotrophic bacterial and phototrophic protistan taxa in CO_2 fixation using the "rfPermut" package. The environmental (climatic and soil variables) and spatial effects on community variations (pairwise Bray–Curtis dissimilarity) were determined by variation partitioning analysis (VPA). Spatial variables were estimated from geographic distances by principal coordinates analysis of neighbor matrices using the "vegan" package.

The co-occurrence networks of *cbbL*-containing autotrophic bacteria and phototrophic protists were constructed in upland, paddy and forest soils. The top ASVs accounting for more than 80% of the relatively abundant community in each ecosystem were selected, among which the robust correlations (Spearman's coefficient >.65 and *p* value <.01) were retained for network construction (Delgado-Baquerizo et al., 2018; Fan et al., 2021). The robustness of the co-occurrence networks was estimated as the proportion of the remaining taxa in the community with 50% of the nodes randomly removed from the network. The main ecological clusters in the empirical network were explored and visualized using Gephi 0.9.2. The relative abundance of each main ecological cluster was computed by averaging the standardized relative abundances (*z* score) of the ASVs within each module.

Structural equation modeling (SEM) was performed to evaluate the direct and indirect effects of climatic (MAT and MAP) and edaphic factors (soil pH, TC, TN and AP) and the main ecological clusters of C fixers on CO_2 fixation rates in different ecosystems using IBM SPSS Amos 21. The standardized total effects (STEs) of the biotic and abiotic factors on C fixation rates were calculated.

3 | RESULTS

3.1 | Carbon fixation rates vary with ecosystem types

Soil CO_2 fixation rates exhibited large variations across 159 soil samples, ranging from 0.185 to 5.899 mg CO_2 soil kg^{-1} day^{-1}, with the highest in paddy soils (2.447 ± 1.096 mg CO_2 soil kg^{-1} day^{-1}), followed by forest soils (0.648 ± 0.310 mg CO_2 soil kg^{-1} day^{-1}) and upland soils (0.583 ± 0.191 mg CO_2 soil kg^{-1} day^{-1}) (Figure 1). The spatial distribution of soil C fixation rates in paddy soils increased significantly from high to low latitudes ($p < .001$; Figure S2). The fixation rates in upland soils increased from 18° N to 27° N and declined at higher latitudes, while the lowest fixation rates appeared at 23°–29° N in forest soils. Soil TC was positively correlated with the CO_2 fixation rates in forest and paddy soils, indicating the crucial contribution of microbial autotrophic processes to soil C sequestration (Figure S3).

3.2 | Distinct profiling of soil autotrophic bacteria and protistan phototrophs across different ecosystems

The α-diversity indices of the *cbbL*-containing autotrophic bacteria and phototrophic protists were significantly higher in paddy soils than in upland and forest soils (Figure S4). Paddy soils also possessed higher absolute abundances of autotrophic bacteria and Chlorophyta than upland and forest soils (Figure S5). NMDS and PERMANOVA results showed a pronounced separation in

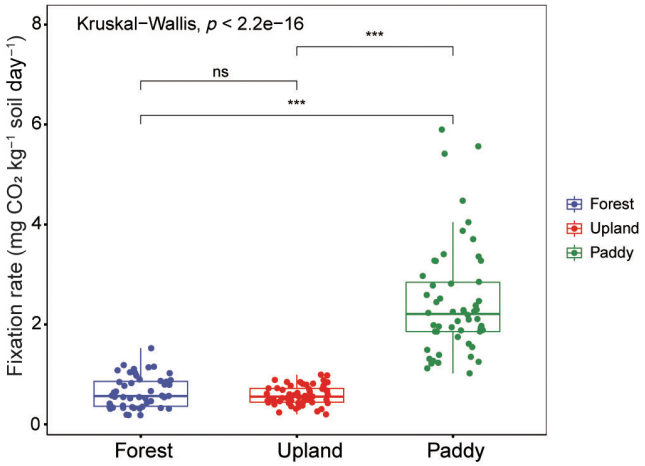

FIGURE 1 Soil microbial CO_2 fixation potentials in terrestrial ecosystems. Boxplot showing the differences in CO_2 fixation rates among forest, upland and paddy ecosystems across the large scale. ***$p < .001$; ns, $p > .05$.

the community composition of the autotrophic bacteria and phototrophic protists between paddy soils and forest/upland soils ($p < .001$; Figure 2a; Table S1). Paddy soils harbored a higher relative abundance of obligate autotrophic bacterial species belonging to Thiobacillaceae and Acidiferrobacteraceae but less facultative autotrophy (such as Pseudonocardiaceae and Phyllobacteriaceae) than other ecosystems (Figure 2b). For the dominant phototrophic protist lineages, the relative abundances of Chlorophyceae, which serve as obligate phototrophs, were significantly higher in upland and paddy soils, while Chrysophyceae were more abundant in forest soils. Both the autotrophic bacteria and phototrophic indicator ASVs were unique in each ecosystem (Figure S6). Consistently, more Chlorophyceae, Thiobacillaceae and Acidiferrobacteraceae indicators were identified in paddy rather than in upland and forest soils.

3.3 | Geographic distribution patterns of autotrophic bacteria and protistan phototrophs

The generalized additive models across the NMDS ordination showed that latitude ($R^2 > .67$) was a strong determinant for autotrophic bacteria and protistan phototrophs in the three ecosystems (Figure 3). Pronounced distance-decay relationships (DDRs) were also detected in autotrophic bacterial and phototrophic protist communities across eastern China spanning a geographic distance of 3689 km (Figure S7). The steeper slopes of autotrophic bacteria and phototrophic protists in paddy soils implied a higher spatial turnover rate of community composition than those in forest and upland soils.

The VPA results revealed comparable impacts of the spatial effects and environmental drivers on the biogeographic patterns of autotrophic bacteria and phototrophic protists (Figure S8). The Mantel test showed that both the edaphic and climate factors

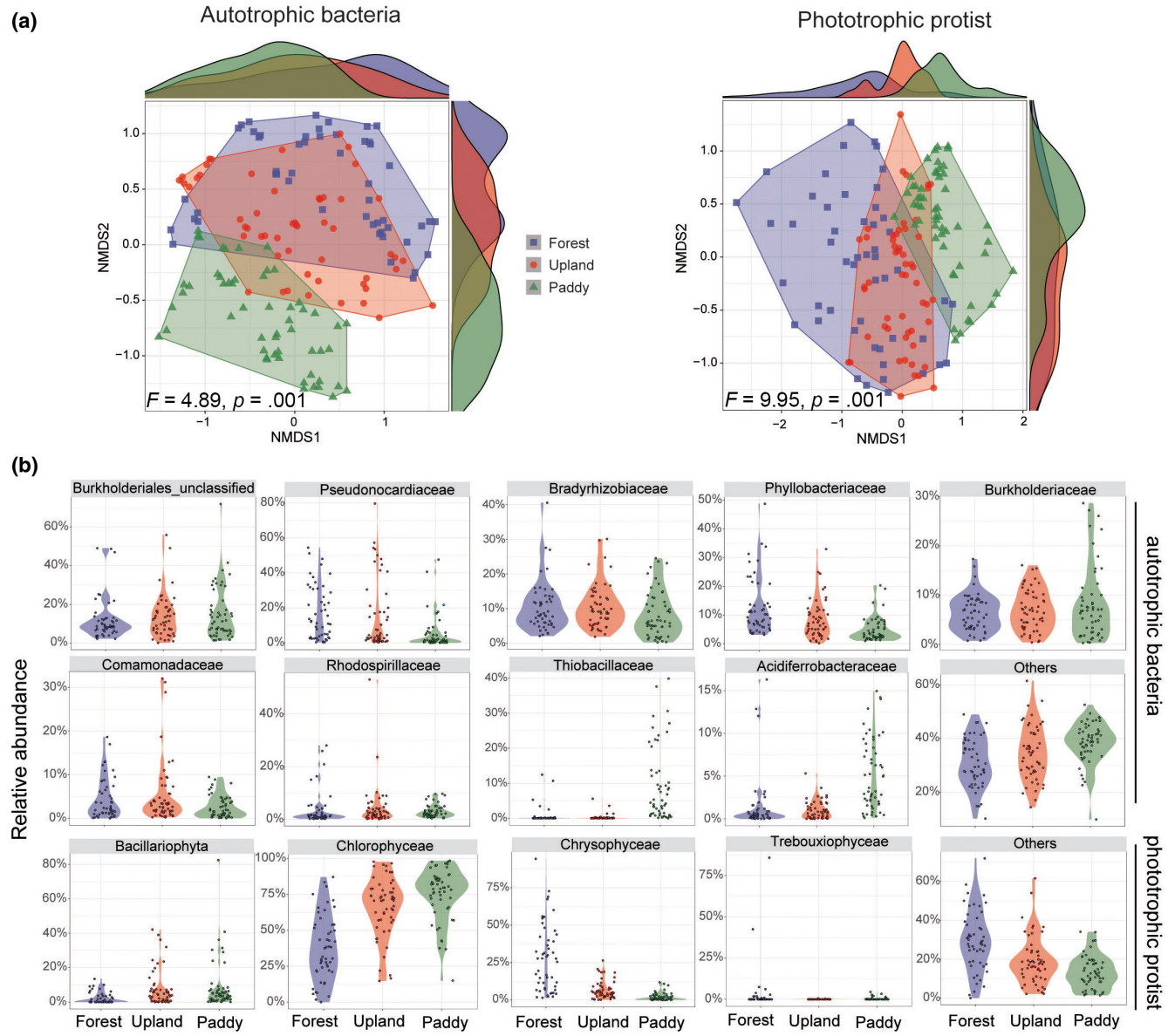

FIGURE 2 Community structures of soil autotrophic microorganisms among forest, upland, and paddy ecosystems. (a) Nonmetric multidimensional scaling showed the structure of soil *cbbL*-containing autotrophic bacteria and phototrophic protists communities in forest, upland, and paddy soils. The effects of ecosystem types on *cbbL*-containing autotrophic bacteria and phototrophic protist communities were examined by permutational multivariate analysis of variance. Curves next to and above the ordinations show the frequency densities for the corresponding axes. (b) Violin plots showing the relative abundances of autotrophic bacteria at the family level and phototrophic protists at the class level in each ecosystem.

significantly controlled the assembly of the autotrophic microorganisms. Specifically, soil pH was identified as the most significant edaphic driver for the autotrophic bacteria and exhibited comparable effects with climate factors (MAT and MAP) in the three ecosystems (Figure 4). However, the significance of environmental drivers shaping the spatial variation of phototrophic protists varied greatly across ecosystems. Climatic factors were more associated with phototrophic protists in paddy and upland soils, while soil pH was more important in forest soils. In addition, variations in phototrophic protists in paddy soils were more influenced by soil C contents than those in forest and upland soils.

3.4 | Autotrophic microorganisms explain the most variation in soil CO_2 fixation

The BRT results showed that the community composition of autotrophic bacteria was the most important factor governing soil CO_2 fixation rates in terrestrial ecosystems, accounting for 57% of the total contributions (Figure 5). Autotrophic bacteria and phototrophic protists together explained 72% of the variation in soil CO_2 fixation, followed by edaphic (26%) and climate (2%) factors. Furthermore, the total relative contributions of edaphic factors to soil CO_2 fixation were predominant in forest (62%) and upland (56%) soils, while

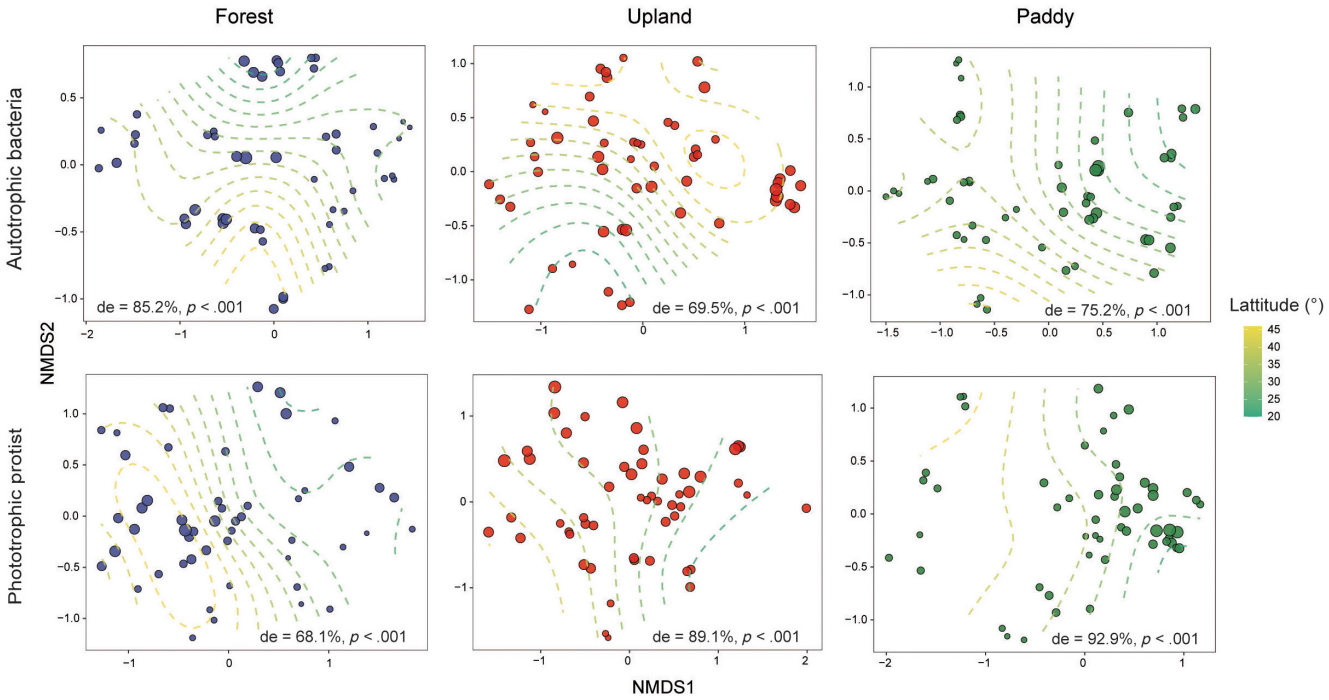

FIGURE 3 Spatial distributions of soil autotrophic microbial communities at the large scale. Nonmetric multidimensional scaling (NMDS) ordination including the output of a generalized additive model for the latitude of the *cbbL*-containing autotrophic bacterial and phototrophic protistan communities in forest, land, and paddy soils. Green splines show the fit from high latitude (light yellow) to low latitude (light green) over the ordination. The size of the points is associated with the CO_2 fixation rates in each ecosystem.

autotrophic microorganisms were more important in paddy soils (53%). In addition, a higher contribution of phototrophic protists to CO_2 fixation was observed in paddy soils than upland and paddy soils.

A random forest model further identified the importance of autotrophic microbial taxa in CO_2 fixation (R^2 = 36.6%–55.2%) (Figure S9). Changes in the relative abundance of Thiobacillaceae and Acidiferrobacteracea explained the variations in CO_2 fixation potential across all samples. Autotrophic bacterial members belonging to Comamonadaceae and Acidiferrobacteracea play crucial roles in CO_2 fixation in forest and upland soils. In contrast, phototrophic lineages such as Chlorophyceae and Trebouxiophyceae were better predictors of CO_2 fixation in paddy soils.

3.5 | Key microbial ecological clusters linking soil CO_2 fixation

The relationships between autotrophic bacterial and phototrophic protistan taxa tended to be co-occurrence rather than exclusion, with the major taxa being positively correlated, especially in paddy soils (Figure 6a). The co-occurrence networks of the autotrophic microorganisms in paddy soils were more complex and clustered than those in upland and forest soils, which was reflected by the remarkably larger number of network nodes and links, as well as a higher modularity and node connectedness (average degree) (Figure 6; Table S2). On the basis of random species loss, the co-occurrence network of the autotrophic microorganisms in paddy soils displayed a significantly higher robustness than the networks in forest and upland soils ($p < .05$), indicating a strengthened resistance (Figure S10).

The main ecological clusters consisted of multiple and diverse autotrophic bacterial and phototrophic protistan species but with distinct proportions of phylotypes in different ecosystems (Figure 6; Figure S11). SEM provided integrated information on the direct and indirect effects of the major ecological predictors on apparent CO_2 fixation rates across different ecosystems (Figure 6). The models explained a large proportion of the variations in CO_2 fixation (75% for forest, 64% for upland and 73% for paddy fields), with a strong goodness of fit. FoMod1 and FoMod2 in forest soils, LaMod3 in upland soil, and PaMod1 and PaMod3 in paddy soils (Figure 6; Figure S11, $p < .05$) were identified as the key clusters, which were directly and positively associated with CO_2 fixation rates based on both SEM and linear regression results.

The variations in abiotic attributes (climate and soil properties) across the large scale indirectly affected CO_2 fixation by altering the relative abundances of key ecological clusters of C fixers (Figure 6). Collectively, the STE summarized both the direct and indirect effects of each ecological predictor and revealed that microbial-driven CO_2 fixation was more affected by soil proprieties (especially soil pH) in forest and upland ecosystems but was more affected by MAP in paddy fields. The latitude effect on CO_2 fixation is stronger in paddy ecosystems.

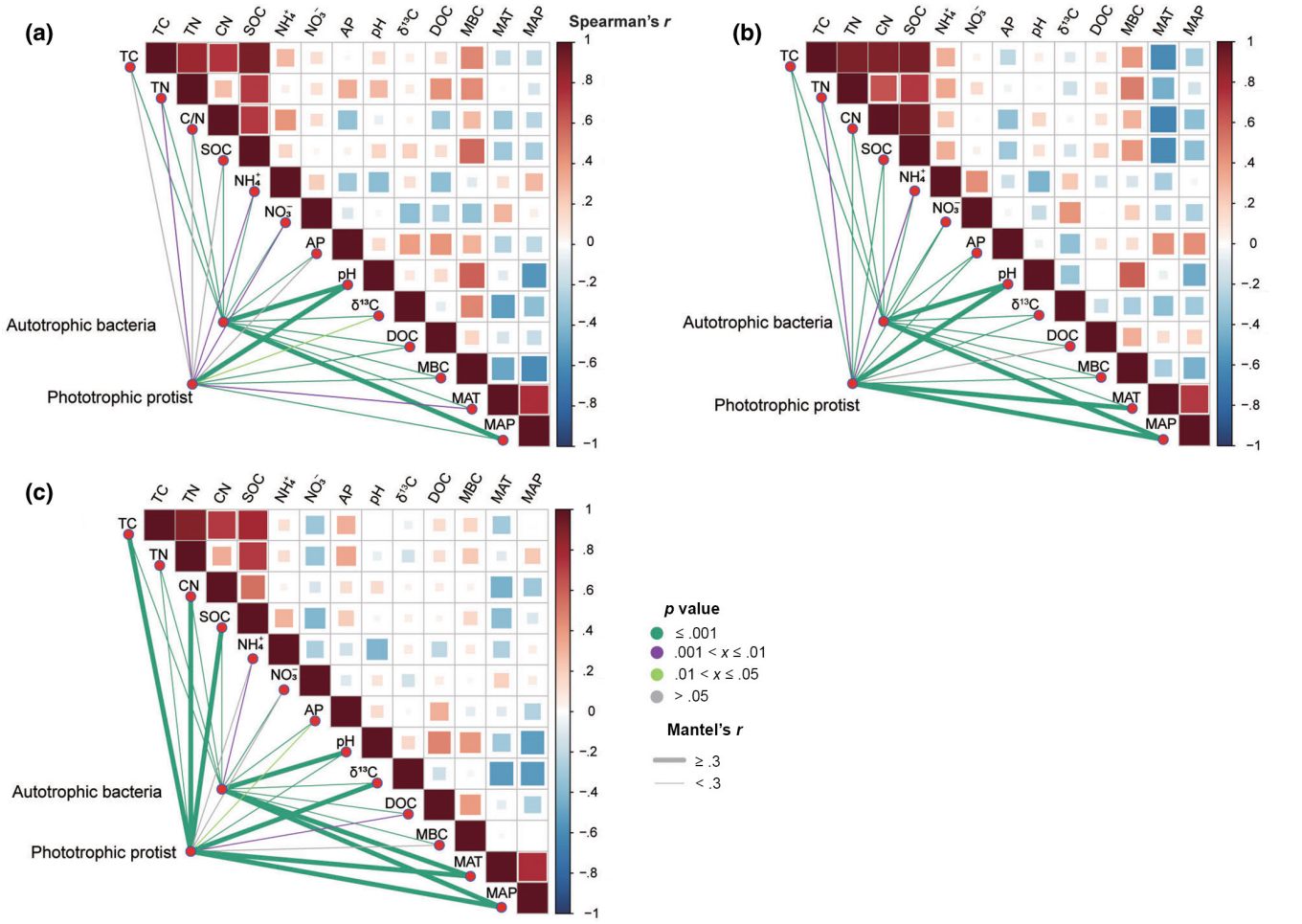

FIGURE 4 Drivers of variation in the autotrophic microorganisms in the terrestrial ecosystem. Mantel analysis examining the relationship between environmental factors and the composition of *cbbL*-containing autotrophic bacterial and phototrophic protistan communities in the forest (a), upland (b), and paddy (c) environments. AP, available phosphorus; C/N, ratio of carbon to nitrogen; DOC, dissolved organic carbon; MAP, mean annual precipitation; MAT, mean annual temperature; MBC, microbial biomass carbon; NH_4^+, ammonium; NO_3^-, nitrate; SOC, soil organic carbon; TC, total carbon; TN, total nitrogen.

4 | DISCUSSION

Recent theoretical advances and local studies have highlighted the roles of soil microbes in C fixation and their contributions to the stable SOC pools (Akinyede et al., 2020; Spohn et al., 2020; Xiao et al., 2021). However, a quantification of the importance of soil autotrophic microbes for explaining soil CO_2 fixation across large environmental gradients, and in combination with other important environmental factors is largely lacking. In this study, we provide large-scale evidence that autotrophic microbes largely explain soil CO_2 fixation patterns in natural and agricultural ecosystems. This knowledge is critical to better understand expectations that plant restoration and photosynthetic processes can mitigate climate change and CO_2 emissions without accounting for soil microbes.

Our results showed that CO_2 fixation rate was significantly correlated with soil TC content in upland and forest soils, apparently indicating the potential contribution of microbial CO_2-fixing processes to the SOC storage. However, CO_2 fixation rate was decoupled with TC in upland soils, possibly due to the weakened contributions of soil CO_2 fixation to SOC by fertilization. This interpretation was supported by our previous study showing that chemical fertilization decreased CO_2 fixation rates by 57% through reducing soil pH in upland soils (Liao et al., 2020). The estimated percentage of annual microbial CO_2 fixation to SOC was ~0.45%, 0.48%, and 1.12% in forest, upland, and paddy soils, respectively. Assuming a soil bulk density of 1.1–1.5 $g\,cm^{-3}$, the autotrophic microbial C input into forest, upland, and paddy surface soils (0–20 cm) will be 142–194, 128–174, and 536–731 $kg\,C\,ha^{-1}\,year^{-1}$, respectively. This part of overlooked C input in soils should be taken into account in earth system models for more accurate prediction of the global carbon cycle.

The large-scale variations in CO_2 fixation rates were highly related to the biogeographic distributions of bacterial and protist autotrophs in different ecosystems. Although the communities of autotrophs were structured along a latitudinal gradient in three ecosystems, the steeper slope of DDRs in paddy soils indicated greater spatial variations in microbial autotrophs than those in forest and dryland soils. A possible explanation is that paddy environments with long-term dry-wet cycling create ephemeral but unique habitats, thus

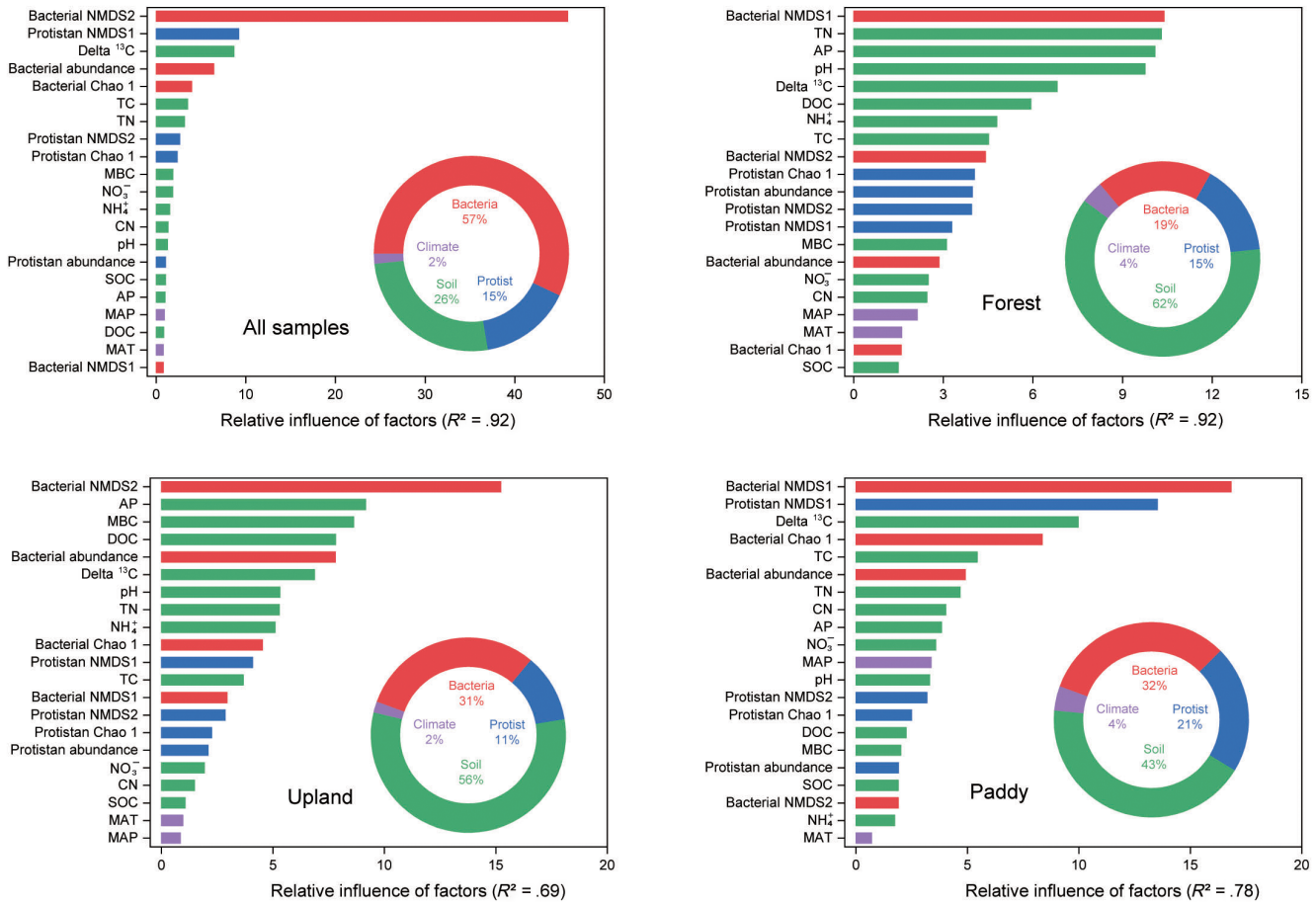

FIGURE 5 Relative influence of different factors on the microbial CO_2 fixation rates across forest, upland and paddy soils. Barplots and donut charts indicate the relative influence of autotrophic bacterial and phototrophic protistan attributes (abundances, diversity, and community composition), soil conditions (soil TC, TN, C/N, SOC, MBC, DOC, NH_4^+, NO_3^-, Delta ^{13}C, AP, and pH), and climatic factors (MAT and MAP). AP, available phosphorus; C/N, ratio of carbon to nitrogen; DOC, dissolved organic carbon; MAP, mean annual precipitation; MAT, mean annual temperature; MBC, microbial biomass carbon; NH_4^+, ammonium; NO_3^-, nitrate; SOC, soil organic carbon; TC, total carbon; TN, total nitrogen.

supporting more spatially structured bacterial and protistan communities across large scales (Hu et al., 2013; Wang et al., 2015). In contrast to previous investigations based on local sites showing the importance of soil properties (such as pH, ammonium nitrogen, and AP) in driving CO_2 fixation by autotrophic bacteria (Liao et al., 2020; Wu et al., 2014; Xiao et al., 2018), MAP in paddy soils and soil pH in forest and upland ecosystems were identified as important ecological predictors for the large-scale microbial CO_2 fixation.

Paddy fields commonly serve as crucial C sinks, partially because the anaerobic conditions retard microbial decomposition process and strengthen microbial necromass accumulation (Bay et al., 2021; Chen et al., 2021). Here, we highlight the direct contribution of microbially mediated CO_2 fixation to SOC pools in paddy soils, which has never been evidenced previously at the large scale. Due to the complex and heterogeneous environment generated by the specific agricultural practices such as frequent flooding, tillage, or intermittent irrigation (Hu et al., 2013; Huang & Hall, 2017; Jiao et al., 2019), paddy fields consistently harbored a higher abundance and diversity of microbial autotrophs, tightened associations, and displayed 4–5 times higher CO_2 fixing rates than upland and forest ecosystems. Notably, enrichment of the obligate autotrophs in paddy soils could enhance CO_2 fluxes to soils, since obligate autotrophs display higher CO_2-fixing efficiencies than facultative autotrophs (Esparza et al., 2010; Ge et al., 2012). Members in Thiobacillaceae and Acidiferrobacteraceae, in particular, can utilize CO_2 as the sole C source by oxidation of sulfur using ferric iron as an electron acceptor under anaerobic conditions and oxidation of ferrous iron using oxygen as an electron acceptor in aerobic environment (Issotta et al., 2018; Monachon et al., 2019). The frequent transformation of iron and sulfur at the oxic–anoxic interface in paddy ecosystem may facilitate the enrichment of autotrophic iron and sulfur oxidizers and accelerate the chemolithoautotrophic fixation of CO_2 (Tong et al., 2021).

As primary producers, the prominent role of phototrophic protists in CO_2 fixation has been well documented in marine ecosystems (Mitra et al., 2014), but is less explored in terrestrial ecosystems. Our study first evaluated the contribution of phototrophic protists to SOC storage within terrestrial ecosystems,

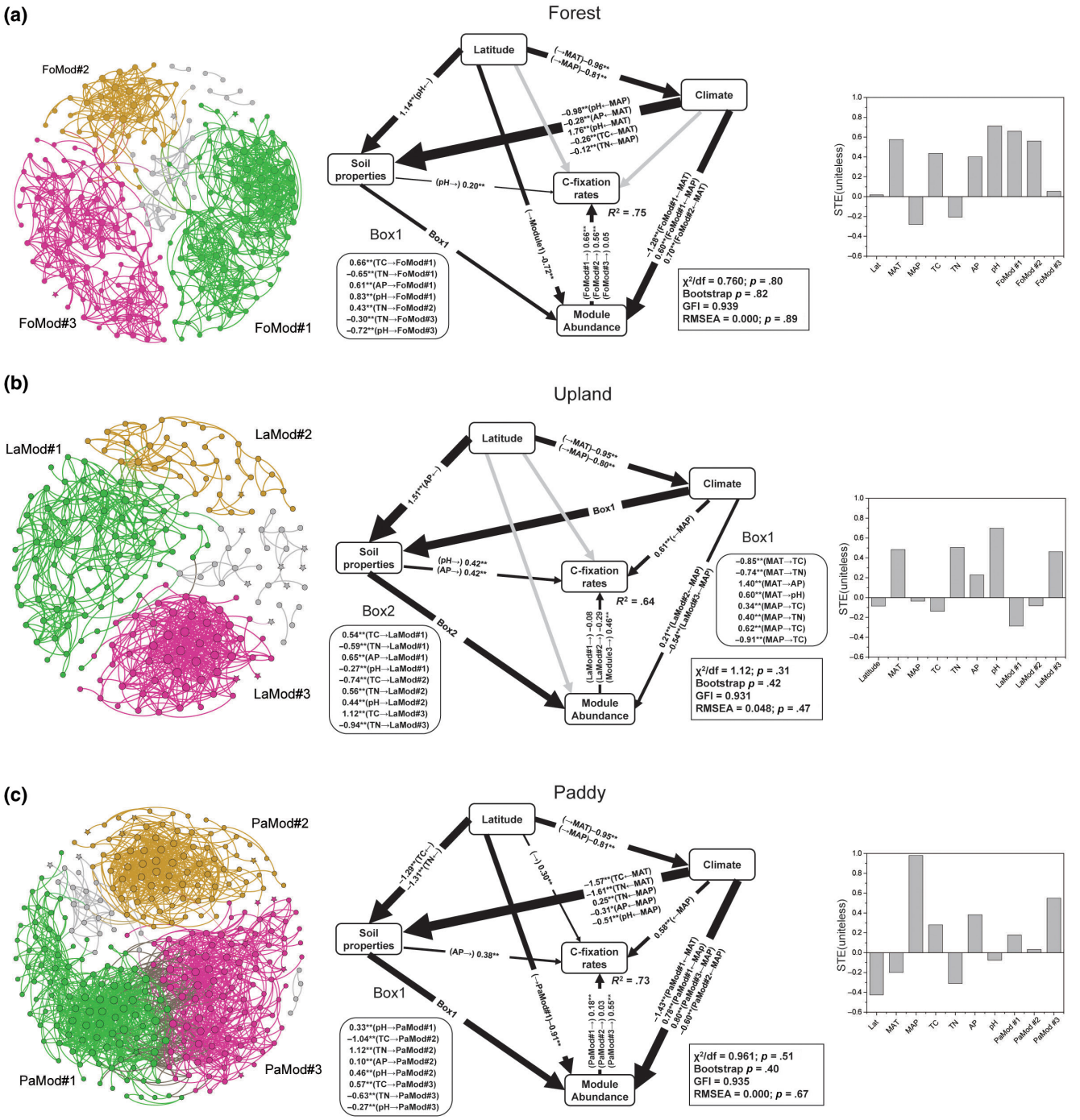

FIGURE 6 The effects of environmental factors and main ecological clusters on soil CO_2 fixation rates. The first column of plots shows the three main ecological clusters of co-occurrence networks in forest (a), upland (b), and paddy (c) ecosystems. Points represent individual autotrophic bacteria, and stars represent protist amplicon sequence variants. The second column of plots shows structural equation modeling (SEM) describing the ecological factors affecting the CO_2 fixation potentials. Soil properties include total carbon (TC), total nitrogen (TN), available phosphorus (AP), and soil pH. Climate includes mean annual temperature (MAT) and mean annual precipitation (MAP). $^*p < .05$ and $^{**}p < .01$. The arrow width is proportional to the strength of the relationship. R^2 appears as the proportion of explained CO_2 fixation rates in the model. The nonsignificant χ^2, nonparametric bootstrap, and the values of root mean square error of approximation and goodness of fit indicate that the model was satisfactorily fitted to the data. The third column of plots shows the standardized total effects (STE) of each factor on the CO_2 fixation rate.

and found up to 21% contribution of phototrophic protists to C storage in paddy soils but less than 15% in forest and upland soils. This finding suggests a higher potential of autotrophic metabolism in water-logged environments such as paddy fields, which was supported by the preference for phototrophic C fixation in aquatic or semiaquatic environments (Stefana et al., 2014). Water content

in soils and bryophytes has been shown to be critical determinant for the survival of phototrophic protists (Jassey et al., 2022). Study by ^{14}C-labelling also confirmed that photosynthetic C fixation was only detected after introducing water in desert soils (Bay et al., 2021). Likewise, simulated rainfall enhanced the activities of photoautotrophs in biocrusts and topsoils (Kidron et al., 2002; Rao et al., 2009). Given the importance of water in governing the survival and activity of photoautotrophs, it is conceivable that photosynthetic process could be enhanced in paddy soils. This is affirmed by our parallel experiment showing significantly higher C fixation rates under flooding than well-drained conditions (40% WHC) in paddy soils (Figure S12).

It should be noted that the CO_2 fixation rates in this study were determined in laboratory without considering the effect of plants. In natural ecosystems, the input of plant-derived C (e.g. root exudates and plant litters) could influence the assemblies of autotrophic microbes and C fixation, since obligate autotrophs tend to proliferate in nutrient-poor environments (Badger & Bek, 2008; Wu et al., 2014) while facultative autotrophs prefer SOC-rich conditions (Akinyede et al., 2020; Xiao et al., 2018). Additionally, the proliferation of fast-growing heterotrophic microbes stimulated by plant-derived C may impact CO_2 fixation through competing with autotrophs for niches and altering the CO_2 concentration in soil pores (Akinyede et al., 2022; Spohn et al., 2020). Thus, the influence of plant-derived C on microbial autotrophic processes needs further investigations.

In conclusion, our study provides the first empirical evidence that autotrophic bacterial and phototrophic protistan communities explain the large-scale variations in the potential of soil microbial CO_2 fixation in forest and agricultural ecosystems. Autotrophic microbial processes are more active in waterlogged ecosystems. The spatial patterns of CO_2 fixation were highly associated with the ecological clusters of the co-occurrence network of autotrophic microbes, which are mainly driven by soil pH in forest and upland environments, and by MAP in paddy ecosystems. This work allows us to have a more in-depth understanding of soil CO_2 fixation in terrestrial ecosystems, and future modeling studies should thus consider autotrophic processes to better predict soil C dynamics.

AUTHOR CONTRIBUTIONS

Qiaoyun Huang, Xiuli Hao, and Hao Liao conceived and designed the project. Hao Liao, Xiuli Hao, and Fei Qin analyzed the data and prepared the figures. Hao Liao and Fei Qin performed lab work. Hao Liao and Xiuli Hao wrote the paper with comments from Qiaoyun Huang, Wenli Chen, Manuel Delgado-Baquerizo, Jizhong Zhou, Yurong Liu, and Peng Cai.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Raw sequence data are available at the NCBI Sequence Read Archive (SRA) database for *cbbL*-containing autotrophic bacteria (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA811373) and protists (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA811386). The data that support the findings of this study are openly available at https://doi.org/10.5061/dryad.2fqz612sk.

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REFERENCES

Akinyede, R., Taubert, M., Schrumpf, M., Trumbore, S., & Kuesel, K. (2020). Rates of dark CO_2 fixation are driven by microbial biomass in a temperate forest soil. *Soil Biology and Biochemistry*, *150*, 107950.

Akinyede, R., Taubert, M., Schrumpf, M., Trumbore, S., & Kuesel, K. (2022). Dark CO_2 fixation in temperate beech and pine forest soils. *Soil Biology and Biochemistry*, *165*, 108526.

Badger, M. R., & Bek, E. J. (2008). Multiple rubisco forms in proteobacteria: Their functional significance in relation to CO_2 acquisition by the CBB cycle. *Journal of Experimental Botany*, *59*, 1525–1541.

Bastida, F., García, C., Fierer, N., Eldridge, D. J., Bowker, M. A., Abades, S., Alfaro, F. D., Asefaw Berhe, A., Cutler, N. A., Gallardo, A., García-Velázquez, L., Hart, S. C., Hayes, P. E., Hernández, T., Hseu, Z. Y., Jehmlich, N., Kirchmair, M., Lambers, H., Neuhauser, S., … Delgado-Baquerizo, M. (2019). Global ecological predictors of the soil priming effect. *Nature Communications*, *10*, 3481.

Bay, S. K., Waite, D. W., Dong, X., Gillor, O., Chown, S. L., Hugenholtz, P., & Greening, C. (2021). Chemosynthetic and photosynthetic bacteria contribute differentially to primary production across a steep desert aridity gradient. *The ISME Journal*, *15*, 3339–3356.

Bond-Lamberty, B., Bailey, V. L., Chen, M., Gough, C. M., & Vargas, R. (2018). Globally rising soil heterotrophic respiration over recent decades. *Nature*, *560*, 80–83.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583.

Carvalhais, N., Forkel, M., Khomik, M., Bellarby, J., Jung, M., Migliavacca, M., Mu, M., Saatchi, S., Santoro, M., Thurner, M., Weber, U., Ahrens, B., Beer, C., Cescatti, A., Randerson, J. T., & Reichstein, M. (2014). Global covariation of carbon turnover times with climate in terrestrial ecosystems. *Nature*, *514*, 213–217.

Chen, X., Hu, Y., Xia, Y., Zheng, S., Ma, C., Rui, Y., He, H., Huang, D., Zhang, Z., Ge, T., Wu, J., Guggenberger, G., Kuzyakov, Y., & Su, Y. (2021). Contrasting pathways of carbon sequestration in paddy and upland soils. *Global Change Biology*, *27*, 2478–2490.

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*, 165–173.

De Caceres, M., Legendre, P., & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos*, *119*, 1674–1684.

Delgado-Baquerizo, M., Reith, F., Dennis, P. G., Hamonts, K., Powell, J. R., Young, A., Singh, B. K., & Bissett, A. (2018). Ecological drivers of soil microbial diversity and soil biological networks in the southern hemisphere. *Ecology*, *99*, 583–596.

Esparza, M., Pablo Cardenas, J., Bowien, B., Jedlicki, E., & Holmes, D. S. (2010). Genes and pathways for CO_2 fixation in the obligate, chemolithoautotrophic acidophile, *Acidithiobacillus ferrooxidans*, carbon fixation in *A. ferrooxidans*. *BMC Microbiology*, *10*, 229.

Fan, K., Delgado-Baquerizo, M., Guo, X., Wang, D., Zhu, Y. G., & Chu, H. (2021). Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. *The ISME Journal*, *15*, 550–561.

Ge, T., Yuan, H., Zhu, H., Wu, X., Nie, S., Liu, C., Tong, C., Wu, J., & Brookes, P. (2012). Biological carbon assimilation and dynamics in a flooded rice - Soil system. *Soil Biology and Biochemistry*, *48*, 39–46.

Guo, L., Deng, M., Yang, S., Liu, W., Wang, X., Wang, J., & Liu, L. (2021). The coordination between leaf and fine root litter decomposition and the difference in their controlling factors. *Global Ecology and Biogeography*, *30*, 2286–2296.

Guo, S., Xiong, W., Hang, X., Gao, Z., Jiao, Z., Liu, H., Mo, Y., Zhang, N., Kowalchuk, G. A., Li, R., Shen, Q., & Geisen, S. (2021). Protists as main indicators and determinants of plant performance. *Microbiome*, *9*, 64.

Hartman, K., van der Heijden, M. G. A., Wittwer, R. A., Banerjee, S., Walser, J.-C., & Schlaeppi, K. (2018). Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome*, *6*, 14.

Hu, H. W., Zhang, L. M., Yuan, C. L., & He, J. Z. (2013). Contrasting Euryarchaeota communities between upland and paddy soils exhibited similar pH-impacted biogeographic patterns. *Soil Biology and Biochemistry*, *64*, 18–27.

Huang, W., & Hall, S. J. (2017). Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter. *Nature Communications*, *8*, 1774.

Hutsch, B. W., Augustin, J., & Merbach, W. (2002). Plant rhizodeposition - An important source for carbon turnover in soils. *Journal of Plant Nutrition and Soil Science*, *165*, 397–407.

Issotta, F., Moya-Beltrán, A., Mena, C., Covarrubias, P. C., Thyssen, C., Bellenberg, S., Sand, W., Quatrini, R., & Vera, M. (2018). Insights into the biology of acidophilic members of the Acidiferrobacteraceae family derived from comparative genomic analyses. *Research in Microbiology*, *169*, 608–617.

Jansson, J. K., & Hofmockel, K. S. (2020). Soil microbiomes and climate change. *Nature Reviews Microbiology*, *18*, 35–46.

Jassey, V. E. J., Hamard, S., Lepère, C., Céréghino, R., Corbara, B., Küttim, M., Leflaive, J., Leroy, C., & Carrias, J. F. (2022). Photosynthetic microorganisms effectively contribute to bryophyte CO_2 fixation in boreal and tropical regions. *ISME Communications*, *2*, 64.

Jiao, S., Xu, Y., Zhang, J., & Lu, Y. (2019). Environmental filtering drives distinct continental atlases of soil archaea between dryland and wetland agricultural ecosystems. *Microbiome*, *7*, 15.

Kidron, G. J., Herrnstadt, I., & Barzilay, E. (2002). The role of dew as a moisture source for sand microbiotic crusts in the Negev Desert, Israel. *Journal of Arid Environment*, *52*, 517–533.

Liao, H., Qin, F., Wang, K., Zhang, Y., Hao, X., Chen, W., & Huang, Q. (2020). Long-term chemical fertilization-driving changes in soil autotrophic microbial community depresses soil CO_2 fixation in a Mollisol. *Science of the Total Environment*, *748*, 141317.

Liu, Y., Ge, T., Zhu, Z., Liu, S., Luo, Y., Li, Y., Wang, P., Gavrichkova, O., Xu, X., Wang, J., Wu, J., Guggenberger, G., & Kuzyakov, Y. (2019). Carbon input and allocation by rice into paddy soils: A review. *Soil Biology and Biochemistry*, *133*, 97–107.

Long, X. E., Yao, H., Wang, J., Huang, Y., Singh, B. K., & Zhu, Y. G. (2015). Community structure and soil pH determine chemoautotrophic carbon dioxide fixation in drained paddy soils. *Environmental Science & Technology*, *49*, 7152–7160.

Mitra, A., Flynn, K. J., Burkholder, J. M., Berge, T., Calbet, A., Raven, J. A., Granéli, E., Glibert, P. M., Hansen, P. J., Stoecker, D. K., Thingstad, F., Tillmann, U., Våge, S., Wilken, S., & Zubkov, M. V. (2014). The role of mixotrophic protists in the biological carbon pump. *Biogeosciences*, *11*, 995–1005.

Monachon, M., Albelda-Berenguer, M., & Joseph, E. (2019). Biological oxidation of iron sulfides. In G. M. Gadd & S. Sariaslani (Eds.), *Advances in applied microbiology* (Vol. *107*, pp. 1–27). Academic Press.

Rao, B., Liu, Y., Wang, W., Hu, C., Dunhai, L., & Lan, S. (2009). Influence of dew on biomass and photosystem II activity of cyanobacterial crusts in the Hopq Desert, Northwest China. *Soil Biology and Biochemistry*, *41*, 2387–2393.

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., Zscheischler, J., Beer, C., Buchmann, N., Frank, D. C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S., Walz, A., & Wattenbach, M. (2013). Climate extremes and the carbon cycle. *Nature*, *500*, 287–295.

Saini, R., Kapoor, R., Kumar, R., Siddiqi, T. O., & Kumar, A. (2011). CO_2 utilizing microbes - A comprehensive review. *Biotechnology Advances*, *29*, 949–960.

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*, 49–56.

Selesi, D., Schmid, M., & Hartmann, A. (2005). Diversity of green-like and red-like ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes (*cbbL*) in differently managed agricultural soils. *Applied and Environmental Microbiology*, *71*, 175–184.

Spohn, M., Mueller, K., Hoeschen, C., Mueller, C. W., & Marhan, S. (2020). Dark microbial CO_2 fixation in temperate forest soils increases with CO_2 concentration. *Global Change Biology*, *26*, 1926–1935.

Stefana, G., Cornelia, B., Joerg, R., & Michael, B. (2014). Soil water availability strongly alters the community composition of soil protists. *Pedobiologia*, *57*, 205–213.

Tolli, J., & King, G. M. (2005). Diversity and structure of bacterial chemolithotrophic communities in pine forest and agroecosystem soils. *Applied and Environmental Microbiology*, *71*, 8411–8418.

Tong, H., Zheng, C., Li, B., Swanner, E. D., Liu, C., Chen, M., Xia, Y., Liu, Y., Ning, Z., Li, F., & Feng, X. (2021). Microaerophilic oxidation of Fe(II) coupled with simultaneous carbon fixation and As(III) oxidation and sequestration in karstic paddy soil. *Environmental Science & Technology*, *55*, 3634–3644.

Wang, B., Zhao, J., Guo, Z., Ma, J., Xu, H., & Jia, Z. (2015). Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. *The ISME Journal*, *9*, 1062–1075.

Wu, X., Ge, T., Yuan, H., Li, B., Zhu, H., Zhou, P., Sui, F., O'Donnell, A. G., & Wu, J. (2014). Changes in bacterial CO_2 fixation with depth in agricultural soils. *Applied Microbiology and Biotechnology*, *98*, 2309–2319.

Xiao, H., Li, Z., Chang, X., Deng, L., Nie, X., Liu, C., Liu, L., Jiang, J., Chen, J., & Wang, D. (2018). Microbial CO_2 assimilation is not limited by the decrease in autotrophic bacterial abundance and diversity in eroded watershed. *Biology and Fertility of Soils*, *54*, 595–605.

Xiao, K. Q., Bao, P., Bao, Q. L., Jia, Y., Huang, F. Y., Su, J. Q., & Zhu, Y. G. (2014). Quantitative analyses of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) large-subunit genes (*cbbL*) in typical paddy soils. *FEMS Microbiology Ecology*, *87*, 89–101.

Xiao, K. Q., Ge, T. D., Wu, X. H., Peacock, C. L., Zhu, Z. K., Peng, J., Bao, P., Wu, J. S., & Zhu, Y. G. (2021). Metagenomic and ^{14}C tracing

evidence for autotrophic microbial CO_2 fixation in paddy soils. *Environmental Microbiology*, *23*, 924–933.

Yuan, H., Ge, T., Chen, C., O'Donnell, A. G., & Wu, J. (2012). Significant role for microbial autotrophy in the sequestration of soil carbon. *Applied and Environmental Microbiology*, *78*, 2328–2336.

Zhao, K., Kong, W., Wang, F., Long, X. E., Guo, C., Yue, L., Yao, H., & Dong, X. (2018). Desert and steppe soils exhibit lower autotrophic microbial abundance but higher atmospheric CO_2 fixation capacity than meadow soils. *Soil Biology and Biochemistry*, *127*, 230–238.

Zhao, Z. B., He, J. Z., Geisen, S., Han, L. L., Wang, J. T., Shen, J. P., Wei, W. X., Fang, Y. T., Li, P. P., & Zhang, L. M. (2019). Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. *Microbiome*, *7*, 33.

Zhao, Z. B., He, J. Z., Quan, Z., Wu, C. F., Sheng, R., Zhang, L. M., & Geisen, S. (2020). Fertilization changes soil microbiome functioning, especially phagotrophic protists. *Soil Biology and Biochemistry*, *148*, 107863.

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