Bacteria are more sensitive to nitrogen fertilizer application in tea plantation soil while fungi are more correlated to tea yield and quality

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Abstract

Aims

Soil in tea plantations is characterised by severe acidification and high aluminium and fluorine content. Applying excessive nitrogen (N) is a common strategy in tea plantations. Fungal and bacterial responses to N fertiliser addition in tea plantations, especially their relationship with tea growth, quality, and soil microbiome composition, remain unclear.

Methods

We performed a field experiment using different N fertiliser application rates for 5 years (2016–2020) in a tea-producing region of China.

Results

Application of excessive N (600 kg ha^{-1} y^{-1}) reduced tea yield and quality. High N application rates (360 and 600 kg ha^{-1} y^{-1}) significantly decreased bacterial and fungal diversity and altered the compositions of bacterial and fungal communities ($P < 0.05$). Fungi were more tolerant than bacteria to soil environmental changes induced by N fertiliser application. Succession of bacterial and fungal communities was mostly driven by pH. Partial least square path modelling suggested that N addition directly influenced the diversity and communities of bacteria and fungi, and indirectly influenced bacterial community and fungal diversity by mediating soil nutrients and pH. The assembly of fungal communities was more regulated by dispersal limitation and deterministic processes than that of bacterial communities. High microbial diversity was not a requirement for tea growth.

Conclusions

Fungi had a greater impact on tea yield and quality than bacteria; therefore, more attention should be given to fungi, which play a stable role in nutrient cycling and organic matter decomposition in tea plantation, eventually favouring tea growth.

Highlights

- Application of excessive N reduced tea yield and quality.
- High N application rates significantly decreased bacterial and fungal diversity.
- Stochastic processes contributed more to bacterial community assembly than to fungal community assembly in tea plantation soil
- Fungi have a greater impact on tea yield and quality than bacteria.

1 Introduction

Tea (*Camellia sinensis*) is mainly distributed in tropical and subtropical areas with acidic soils (Yan et al., 2018). The unique flavour and health benefits of tea have increased its demand, leading to an expansion of tea-farming area (FAO, 2019; Musial et al., 2020). As a leaf-harvest crop, tea requires more nitrogen (N) to meet the demand for leaf growth and metabolite synthesis than cereal crops, such as maize, rice, and wheat (Tang et al., 2020). Chemical fertiliser application is a practical method for improving tea yield and quality (Wang et al., 2020). In practice, excessive fertiliser (especially N fertiliser) is applied to tea plantations (Ni et al., 2019; Tang et al., 2021b). In China, the average N fertiliser application rate is 491 kg ha^{-1}; excessive N fertiliser application results in soil acidification, eutrophication, and nitrous oxide emissions in 32.2% of tea plantations (Guo et al., 2010; Huang et al., 2017; Wang et al., 2018b; Ni et al., 2019). Moreover, excessive N fertiliser application inhibits theanine and polyphenol synthesis, which contributes to a bitter taste and inferior quality of tea (Ruan et al., 2007; Ruan et al., 2010).

Soil bacteria and fungi play vital roles in maintaining soil productivity, including nutrient cycling, organic matter decomposition, and soil structure composition (Bahram et al., 2018; Kuypers et al., 2018; Naylor et al., 2020). Studies have focussed on bacterial and fungal responses to N addition in terrestrial ecosystems (Geisseler and Scow, 2014; Li et al., 2020b). In arable soils, bacterial and fungal communities are affected when the physicochemical and structural properties of soil are altered by farming practices, such as irrigation, residue return, and fertiliser application (Geisseler and Scow, 2014; de Vries et al., 2018; Chen et al., 2021). Bacteria and fungi have different niches and community assemblies in soil and respond differently to environmental changes. (Schneider et al., 2012; Reay et al., 2019; Jiao et al., 2021a; Zheng et al., 2021). In soil, bacterial networks are more fragile than fungal networks under drought conditions (de Vries et al., 2018). The fungi to bacteria ratio decreases with an increase in the N fertiliser application rate (Zhang et al., 2018). Bacterial beta diversity and the stochasticity ratio first increase and then decrease with an increasing N fertiliser rate, whereas fungal beta diversity is not influenced and the stochasticity ratio decreases with an increasing N fertiliser rate in temperate steppes (Liu et al., 2021). However, a study on temperate grasslands suggested that the fungal community is more sensitive to N addition than the bacterial community (Widdig et al., 2020).

Tea plantation soil is highly acidic; the characteristics high aluminium and fluorine content shape the unique soil microbial community of tea plantations (Shu et al., 2003; Hu et al., 2017; Ji et al., 2018a). Therefore, in the context of global soil acidification, tea plantation soil is optimal for studying the impact of acidification and soil microbial characteristics under highly acidic conditions (Guo et al., 2010). In a subtropical tea plantation, soil fungal community structure was shown to be significantly altered and fungal diversity decreased under higher N input due to a shift in soil and pruning characteristics (Yang et al., 2019). Organic substitution for synthetic N fertiliser can also shift the characteristics of bacterial and fungal communities in tea plantations (Ji et al., 2018b; Ji et al., 2020). However, these studies focused only on soil bacteria and fungi individually. The effects of N addition on soil bacteria and fungi in tea plantations, especially their association with tea yield and quality, remain unclear.

Investigating the assembly process of the microbial community can provide new insights into microbial community succession and assist in exploring microbial function (Stegen et al., 2013; Zhou and Ning, 2017). The soil microbe assembly process is significantly correlated with soil properties, climate change, and land-use history (Zhang et al., 2016; Li et al., 2018; Shi et al., 2018). However, to our knowledge, the assembly process of the microbiome distributed in tea plantation soil, especially the effect of N fertiliser application on microbial assembly, has not been investigated.

In this study, a field experiment with different N fertiliser application rates was commenced in 2016 in a tea-producing area of China. Tea and soil samples were collected in 2020 to evaluate the following: (1) bacterial and fungal community succession under N fertiliser application conditions, (2) factors driving microbial community, (3) consistency of assembly processes of bacteria and fungi under N fertiliser application, and (4) relationship between bacteria and fungi with tea yield and quality.

2 Material And Methods

2.1 Field experiment design

The field experiment was conducted in Shaoxing City, Zhejiang Province, China (29°5616"N, 120°4145"E) during 2016–2020. The experimental site is located in a subtropical monsoon climate region with an annual average temperature of 16.4°C and mean annual precipitation of 1,300 mm. The soil is Haplic Acrisol, according to the Food and Agriculture Organization standards. The initial physicochemical characteristics of soil were pH 3.98, 2.75% soil organic carbon (SOC), 0.17% total nitrogen (TN), 146.04 mg kg^{-1} alkali-hydrolysable N (AN), 182.67 mg kg^{-1} available phosphorus (AP), and 75.78 mg kg^{-1} NH_4OAc-K (AK). The experimental treatments were set as follows: N0 (N deficiency treatment: 0 kg N ha^{-1} y^{-1}), N1 (low N application rate: 120 kg N ha^{-1} y^{-1}), N2 (optimal N application rate: 360 kg N ha^{-1} y^{-1}), and N3 (excessive N application rate: 600 kg N ha^{-1} y^{-1}) (Ruan et al., 2020). P_2O_5 and K_2O application rates were 80 and 100 kg ha^{-1} y^{-1}, respectively, for all treatments. Urea, single superphosphate, and potassium sulphate were selected as N, P, and K fertilisers, respectively. N fertiliser was applied as a base fertiliser at the rate of 30% for 2 months after autumn tea harvest, 30% for 1 month before spring tea harvest, 20% for 1 month before summer tea harvest, and 20% for 1 month before autumn tea harvest. P and K fertilisers were applied as base fertilisers for 2 months after the autumn tea harvest. All fertilisers were applied at a soil depth of 15 cm between tea rows and covered with soil. The four treatments were conducted in a 2 × 18 m^2 experimental plot with three replicates for each treatment based on a randomised block design.

2.2 Tea sampling and analysis

Tea leaves with one bud and two young expanding leaves were harvested in April, July, and September 2020 as spring, summer, and autumn teas, respectively. Tea yield was determined using a 30 × 30 cm stainless-steel frame, and adjusted to 75% of water content. The tea samples were processed for enzyme inactivation immediately after harvest. Subsequently, the samples were oven-dried at 70°C for 48 h before

being sieved through a 250-μm screen. Finally, dry samples were extracted with boiling water to evaluate tea quality. Samples were incubated with ninhydrin to calorimetrically quantify the total amount of free amino acids (AAs) in tea leaves at 570 nm using a spectrophotometer (UV-1780, Shimadzu, Japan). Samples were incubated with the Folin–Ciocalteu reagent for quantifying tea polyphenols at 765 nm using a spectrophotometer (UV-1780, Shimadzu, Japan) (Tang et al., 2021b).

2.3 Soil sampling and analysis of physicochemical characteristics

Soil samples from topsoil (0–25 cm) and subsoil (25–50 cm) were collected after the spring tea harvest in April 2020. The soil samples were divided into two parts: the first was air-dried to determine its physicochemical properties and the second was stored at − 80°C for the analysis of soil bacteria and fungi. The pH of soil extracted with deionised water (soil:deionised water = 1:5) was measured using the Delta 320 pH meter (Mettler-Toledo Instruments Co., Shanghai, China). $K_2Cr_2O_7$ oxidation–reduction titration, Kjeldahl digestion, and alkaline diffusion were used to determine the SOC, TN, and AN, respectively. Soil samples were extracted with 2 M KCl (soil:KCl solution = 1:10) and soil NH_4^+–N and NO_3^-–N concentrations were analysed using a continuous flow analyser (SAN++, Skalar, The Netherlands). Soil AP was extracted using the HCl–NH_4F solution (soil:HCl–NH_4F solution = 1:10) and determined calorimetrically at 700 nm using the molybdenum blue method. Soil samples were extracted with CH_3COONH_4 solution (soil:CH_3COONH_4 = 1:10) to evaluate AK using a flame photometer (INESA 6400A, INESA, Shanghai, China).

2.4 Soil DNA extraction and sequencing of bacteria and fungi

Soil DNA was extracted from 0.5-g soil samples using the FastDNA Spin Kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. The NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to analyse the concentration and quality of the extracted DNA. The V4–V5 regions of 16S rRNA genes were amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') (Tang et al., 2021a). Primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the ITS1 region. Sequencing of polymerase chain reaction products was performed using the Illumina Novaseq platform. Bacterial and fungal sequence data were processed using an in-house pipeline (http://mem.rcees.ac.cn:8080). Same operational taxonomic units were clustered at 97% similarity. Annotation of taxonomic data for representative sequences of bacteria and fungi were processed using the SILVA (https://www.arb-silva.de/) and UNITE (version 8.0, https://unite.ut.ee) databases, respectively.

2.5 Statistical analysis

All data analyses were performed using R (version 4.0.5). The Shapiro–Wilk test was used to assess normality prior to testing significance. Differences in soil and tea characteristics between treatments were

analysed using one-way analysis of variance (ANOVA) and the least significant difference test ($P < 0.05$). Interactions between harvest seasons and treatments were investigated using two-way ANOVA. Random forest analysis was performed using the 'randomForest' package in R. Differences in bacterial and fungal community structures were analysed using non-metric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarities. Canonical redundancy analysis (RDA) and Mantel's test were implemented using the 'vegan' package in R to investigate the contribution of soil properties to bacterial and fungal communities. Partial least squares path modelling (PLS-PM) was performed using the 'plspm' package in R to investigate the relationships between N fertiliser application rate, soil characteristics, and bacterial and fungal community diversity. The contributions of neutral processes to the assembly of bacterial and fungal communities in soil were evaluated using the neutral community model (NCM) proposed by Sloan et al. (2006). In this model, R^2 represents the overall goodness-of-fit of the neutral community model. The Nm, which indicates the migration rate between microbial communities, was calculated by multiplying the metacommunity size and immigration. The parameter m indicates the migration rate (Burns et al., 2016; Chen et al., 2019).

3 Results

3.1 Effect of N fertiliser application on the physicochemical characteristics of soil

N addition altered the physicochemical characteristics of topsoil and subsoil (Table S1). Overall, soil nutrients distributed in the subsoil were present at lower concentrations than in the topsoil. Under N2 and N3 conditions, a significant decrease in soil pH and SOC content was observed in the topsoil compared with that under N0 conditions ($P < 0.05$), whereas there was no significant difference in soil pH between different N application rates in the subsoil. SOC content under N3 conditions in the topsoil and subsoil decreased by 31.8% and 38.4%, respectively, compared with that under N0 conditions. Soil TN and AN decreased as the N fertiliser application rate increased. Under N3 conditions, TN and AN in topsoil decreased by 32.9% and 19.8%, respectively, compared with under N0 conditions. N addition increased soil NH_4^+–N and NO_3^-–N contents and decreased soil AP and AK contents. Nevertheless, 5 years of N fertiliser application did not significantly alter soil C/N in the topsoil or subsoil. Pearson's correlation analysis results indicated a significant negative correlation between soil NO_3^-–N content and soil pH (Fig. S1). A significantly positive correlation was observed between SOC and soil TN, AN, AP, and AK.

3.2 Tea yield and quality under different N fertiliser application rates

Compared with under N0 conditions, tea yields significantly increased in spring, summer, and autumn under N2 and N3 conditions ($P < 0.05$) (Fig. 1A). In all harvest seasons, tea yield increased with N application rate increasing until the N application rate > 360 kg ha^{-1} y^{-1}. Annual yield was defined as the total yield of spring, summer, and autumn tea. Under N1, N2, and N3 conditions annual tea yield

increased by 16.4%, 37.0%, and 33.6%, respectively, compared with under N0 conditions. The results of two-way ANOVA analysis indicated that the N fertiliser application rate and the harvest season had significant effects on tea yield.

Figure 1. Tea yield and quality under different N fertiliser rates in spring, summer, and autumn. Standard deviations of three replicates are represented as error bars. Different letters above the bars indicate significant differences ($P < 0.05$) between N fertiliser treatments in the same harvest season. N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha^{-1} y^{-1} for 5 years.

Under N1, N2, and N3 conditions, AA content significantly increased ($P < 0.05$) by 11.6%, 17.2%, and 17.2%, respectively, for spring tea compared with that under N0 conditions, whereas the N fertiliser application rate did not have a significant effect on the AA content in summer or autumn tea (Fig. 1B). When the N fertiliser application rate was > 360 kg ha^{-1}, the AA content no longer increased with an increase in the N fertiliser application rate. For all harvest seasons, the highest TP content was observed under N0 conditions (Fig. 1C). Conversely, under N2 conditions, the lowest TP content was found in all teas, except for spring tea. Average TP contents of all N application rates were 14.9%, 19.7%, and 27.8% for spring, summer, and autumn, respectively. N addition significantly decreased TP/AA in spring tea compared with that under N0 conditions (Fig. 1D). The lowest TP/AA ratio was observed under N2 conditions for summer and autumn tea. Compared with under N0 conditions, TP/AA under N2 conditions decreased by 26.3%, 25.6%, and 15.6% in spring, summer, and autumn, respectively. Two-way ANOVA results suggested that the N fertiliser application rate and harvest season had significant effects on AA, TP, and TP/AA. However, the interactions between N fertiliser application and harvest season had no significant effect on AA and TP contents or TP/AA.

3.3 Bacterial and fungal community succession under N fertiliser application

The alpha diversities of the bacterial communities decreased with increasing N application rates (Fig. 2A and 2B). Excessive N fertiliser application significantly decreased fungal Chao1 index of topsoil ($P < 0.05$) (Fig. 2C). Two-way ANOVA analysis suggested that N application significantly altered the alpha diversities of bacterial and not fungal communities (Fig. 2F). The soil sampling layer had a significant effect on the Chao1 index of the fungal community. Pearson's correlation analysis results also indicated that the alpha diversities of soil bacteria were more sensitive to soil physicochemical characteristics than soil fungi. Chao1 and Shannon indices of soil bacteria were positively correlated with soil pH, SOC, TN, and AK contents ($P < 0.05$). However, only soil NH_4^+—N and AN content were negatively correlated with the Chao1 and Shannon indices of the fungal community, respectively.

Figure 2. Chao1 and Shannon indices of (A, B) bacterial and (C, D) fungal communities under different N fertiliser application rates. Standard deviations of three replicates are represented as error bars. Different letters above the bars indicate significant differences ($P < 0.05$) between N fertiliser treatments under the same soil layer (topsoil: 0–25 cm, subsoil: 25–50 cm). N0, N1, N2, and N3 refer to N fertiliser application

rates of 0, 120, 360, and 600 kg ha^{-1} y^{-1}, respectively, for 5 years. (E) Pearson's correlation between soil properties and alpha diversity of bacteria and fungi. Red and blue boxes indicate positive and negative correlations between alpha diversity index and soil properties. (F) Two-way ANOVA analysis results; *P< 0.05, **P< 0.01, and ***P< 0.001. SOC: soil organic carbon; TN: total nitrogen; AN: alkali-hydrolysable N; AP: available phosphate; AK: available potassium.

N addition altered the relative abundances of the dominant bacterial and fungal phyla present in soil (Fig. 3). The phyla Proteobacteria, Acidobacteria, Chloroflexi, and Actinobacteria accounted for up to 75% of soil bacteria (Fig. 3A) Basidiomycota, Mortierellomycota, and Ascomycota contributed to up to 35% of soil fungi (Fig. 3C). The relative abundance of Proteobacteria increased with increasing N application rates. The top 10 phyla of soil bacteria were more sensitive to N fertiliser application and the sampling layer than those of soil fungi (Fig. 3B and 3D). Results from Pearson's correlation analysis suggested that the dominant bacterial phyla were more sensitive to soil physicochemical properties than dominant fungal phyla (Fig. 3B and 3D). The phyla Acidobacteria, Actinobacteria, Planctomycetes, and Firmicutes were positively and Proteobacteria and WPS-2 were negatively correlated with soil nutrient content. The fungal phyla Cercozoa and Zoopagomycota were negatively and Mortierellomycota and Rozellomycota were positively correlated with soil nutrient content. NMDS results indicated that soil bacterial and fungal community structures were significantly altered after N addition (Fig. 4A and 4B). Bacterial and fungal community structures distributed in the topsoil and subsoil were significantly different (P< 0.05), and were significantly correlated with soil pH (P< 0.05) (Fig. 4C and 4D).

3.4 Driving factors and assembly of soil bacterial and fungal communities

RDA results revealed that soil pH, SOC, AN, AK, NH_4^+—N, and NO_3^-—N were the main factors driving the bacterial community (Fig. 5A). The soil fungal community was significantly correlated with soil pH, SOC, C/N, AK, NH_4^+—N, and NO_3^-—N (Fig. 5B). Mantel's analysis showed that soil pH, SOC, TN, AN, and AK had stronger correlations with the bacterial community than with the fungal community (Fig. 5C).

However, the soil fungal community was more sensitive to soil NH_4^+—N and AP contents. Results from PLS-PM analysis also suggested that bacterial and fungal diversity was negatively correlated with N fertiliser application (P< 0.05), whereas bacterial and fungal communities were positively correlated with the N fertiliser application rate (P< 0.05) (Fig. 6). N addition influenced the bacterial community by indirectly altering the soil conditions (SOC, TN, and AK) and pH.

A higher R^2 of subsoil suggested that bacteria and fungi distributed in subsoil fitted the NCM better than that in the topsoil, which indicated that the stochastic processes of bacterial and fungal communities increased with the increase in the soil depth (Fig. S2). The migration rates of bacterial and fungal communities in the subsoil were higher than those in the topsoil. Overall, compared with the fungal community, the bacterial community was shaped more by stochastic processes. The migration of the

fungal community was more restricted than the bacterial community. Cluster analysis classified N0 and N1 samples of topsoil and subsoil into one category and N2 and N3 of topsoil into another category, according to soil physicochemical properties (Fig S3). To investigate the impact of N on bacterial and fungal community assemblies, N0 and N1 samples were clustered into a low N fertiliser application rate group, whereas N2 and N3 samples were clustered into a high N fertiliser application rate group (Figs. S4 and S5). The migration rates of bacterial and fungal communities were restricted after high N fertiliser application compared to low N fertiliser application.

3.5 Random forest analysis of tea yield and quality

Random forest analysis was applied to predict the driving factors of tea yield and quality (Fig. 7). The results suggested that the variation in tea yield was mainly driven by beta and alpha diversities of bacterial and fungal communities (Fig. 7A). The most correlated factor for tea yield was the beta diversity of the fungal community. Tea quality was most associated with soil AK; the correlation between tea quality and fungal beta diversity was higher than that with bacterial beta diversity (Fig. 7B). Moreover, Pearson's correlation analysis results suggested that microbial alpha diversity was significantly negatively correlated with tea yield, whereas no significant correlation between microbial alpha diversity and tea quality was observed (Fig. 7).

4 Discussion

4.1 Effect of N input on soil and tea characteristics

Soil physicochemical properties were markedly altered after N addition (Table S1). N2 and N3 conditions affected soil properties more significantly than N1 conditions (Fig. S3). In addition, the effects of N input on topsoil were more apparent than those on subsoil. The decrease of soil pH after N input was related to the release of Al ions (Ruan et al., 2006). Interestingly, after excessive N input, SOC and soil TN decreased simultaneously to maintain a stable C/N ratio, since N addition caused a stoichiometric imbalance and decreased the microbial C utilization efficiency, which stimulated SOC decomposition (Li et al., 2021a; Li et al., 2021b). The soil microbiome regulated stoichiometric stability after nutrient addition (Ma et al., 2021).

Excessive N addition decreased the yield and quality of tea (Fig. 1). These results suggested that highest tea yield and quality could be achieved under N2 conditions. In practice, the N fertiliser application rate under N2 conditions is recommended for green tea production (Ruan et al., 2020). N1 conditions could not meet the N requirement for tea growth. Overuse of N fertiliser decreases soil pH and causes adverse soil conditions (Table S1), which inhibit the synthesis of biochemical components, thereby decreasing tea yield (Ruan et al., 2007; Ma et al., 2013). For instance, arginine and not theanine forms when excessive N fertiliser is applied, resulting in a bitter taste and decreased quality of tea (Ruan et al., 2007). TP/AA is a comprehensive indicator, negatively correlated with green tea quality (Li et al., 2020a). N fertiliser application contributed to the synthesis of AA and decreased the TP content, thereby decreasing TP/AA (Fig. 1). Spring tea was of a higher quality than summer or autumn tea (Fig. 1D), owing to the optimal

spring climate accompanied with suitable temperature and sufficient rain that favour tea growth (Wang et al., 2011); long-term nutrient accumulation from basal fertiliser application to spring tea harvest also contributes to the synthesis of quality ingredients (Sun et al., 2019).

4.2 Succession of bacterial and fungal community under N fertiliser application

Microbial diversity is related to the soil nutrient cycle and contributes to the maintenance of soil function (Zheng et al., 2019; Jiao et al., 2021a). The decrease in bacterial diversity was more apparent than that of fungal diversity with N fertiliser application so as to benefit fungi. This could be explained by the niche differentiation between bacteria and fungi related to their different responses to soil character changes induced by N fertiliser application (Fig. 2E), which has also been demonstrated in previous studies (Dai et al., 2018; Wang et al., 2018a; Liu et al., 2021). Generally, the Chao1 index was more sensitive than the Shannon index under N fertiliser application conditions (Fig. 2). The Chao1 index accounts for species richness and reflects rare species change, whereas the Shannon index suggests both evenness and abundance of species (Shannon, 1948; Chao, 1984). Therefore, the decrease in the fungal Chao1 index indicated that more rare species disappeared, whereas the evenness and abundance of species distributed in the fungal community were not altered under N fertiliser application conditions.

The distribution of the major phyla of both bacteria and fungi was altered by N fertiliser application (Fig. 3). Overall, disturbance of the microbiome was more apparent in the topsoil than in the subsoil; topsoil is more susceptible to farmer practice, animal disturbance, and climate change than subsoil (Jiao et al., 2021a). The bacterial phyla Proteobacteria and Actinobacteria and fungal phylum Ascomycota benefitted from N fertiliser application, whereas the relative abundances of Acidobacteria and Basidiomycota decreased after N addition (Fig. 3A and 3C). These results are consistent with the oligotroph–copiotroph theory (Fierer et al., 2007; Yao et al., 2017). Proteobacteria, Actinobacteria, and Ascomycota are classified as copiotrophic taxa, whereas Acidobacteria and Basidiomycota are oligotrophic taxa according to the oligotroph–copiotroph theory, which is based on the net carbon mineralisation rate of soil (Fierer et al., 2007). Changes of oligotrophic and copiotrophic taxa under N fertiliser application conditions contributed to SOC mineralisation, which led to a decrease in the SOC content (Table S1). The shift in the relative abundance of the dominant phyla was attributed to the increase in N supply, which met the higher N demand of copiotrophic bacteria than that of oligotrophic bacteria (Fierer et al., 2007). Moreover, under N fertiliser application conditions, tea production and organic carbon levels increased, which might also have contributed to changes in the dominant phyla (Fierer et al., 2007; Fierer et al., 2012). The microbial community was significantly influenced by N fertiliser application and soil sampling layer ($P < 0.05$); soil bacteria were more sensitive to the sampling layer (Fig. 4A and 4B). Soil pH was the most important factor regulating both bacterial and fungal communities (Fig. 4C and 4D). Soil acidification induced by N fertiliser application imposes strong environmental filtering, which leads to microbial community assembly through deterministic processes (Leff et al., 2015; Glassman et al., 2017; Tripathi et al., 2018). Similar to the diversity and dominant phyla responses of bacteria and fungi to changes in soil physicochemical characteristics (Fig. 3B and 3D),the

results shown in Fig. 5C indicate that bacterial communities were more associated with soil properties than fungal communities. Studies have shown that fungi are more tolerant to adverse environments, including soil acidification (Rousk et al., 2010) and drought (de Vries et al., 2018) than bacteria. Compared with fungi, the pH range suitable for bacterial growth is narrower (Rousk et al., 2010).

Overall, the NCM results indicated that the fungal community migration rate (m = 0.025) was much lower than that of the bacterial community (m = 0.459) (Fig. S2). Previous studies also demonstrated that dispersal limitation has a greater impact on fungi than bacteria because of their larger size (Schmidt et al., 2014; Chen et al., 2020; Liu et al., 2021). Moreover, the increase in the stochastic process and migration rate of both bacteria and fungi from topsoil to subsoil may be partly explained by the alleviation of subsoil acidification, which affects microbial community distribution (Tripathi et al., 2018). In this study, we found that the fungal stochastic process increased, whereas richness decreased, with an increasing rate of N application (Figs. 2 and S5). Jiao et al. (2021b) also reported that fungal richness decreased with increasing stochastic process, and thus influenced ecosystem functions driven by biodiversity (Jiao et al., 2021a). In tea plantation, assembly process of fungi was more susceptible to N fertiliser application than that of bacteria (Figs. S4 and S5).

4.3 Relationship between bacteria, fungi, and tea

The soil microbiome participates in the nutrient cycle and contributes to plant growth (Saleem et al., 2019). In this study, fungal and bacterial communities and diversity were the most relevant indicators of tea yield. Fungi were more associated with tea yield and quality than bacteria (Fig. 7A and 7B). K plays a vital role in the synthesis of caffeine, AAs, and water-extractable dry matter; thus, soil AK is closely related to tea quality (Fig. 7B) (Ruan et al., 2013). Fungi and bacteria play vital roles in pathogen defence and soil organic matter mineralisation, which leads to a release of available nutrients, thus favouring plant growth (Saleem et al., 2019; Jiao et al., 2021a). Microbial diversity contributes to abiotic and biotic stress resistance, multiple functions, nutrient cycling, and plant productivity because of selection effects, complementarity, or redundancy (Saleem et al., 2019). However, we found that microbial alpha diversity is negatively correlated with tea yield (Fig. 7D and 7F). Although N fertiliser application supplied nutrients that contribute to tea growth, it caused an increase in soil acidification, thereby decreasing microbial alpha diversity. A study also reported that microbial diversity is not always positively correlated with function and productivity (Zhang et al., 2019). Yang et al. (2019) reported that the fungal Chao1 index was negatively correlated with tea pruning biomass. In other words, microbial diversity is not a requirement for the perfect outcome of microbial community because of functional redundancy (Shade, 2017; Louca et al., 2018). In tea plantations, various amounts of pruning litter fall onto the soil surface every year, resulting in the availability of large amounts of nutrients for the microbiome (Yang et al., 2019; Tang et al., 2021a). The possible reason for the close connection between tea and fungi may be that fungi are the initial consumers of plant C input to the rhizosphere (root exudates) (Ballhausen and de Boer, 2016) and soil surface (pruning litter) (Yang et al., 2019). Fungi tend to decompose recalcitrant SOC, such as lignin and cellulose, and bacteria utilise fungal-derived products (de Boer et al., 2005). This decomposition-niche differentiation between fungi and bacteria contributes to nutrient cycling. Moreover,

fungi were more tolerant to adverse environments, including N-induced acidification, than bacteria, thus playing a more stable role under N fertiliser application conditions (Figs. 2, 3, and 5).

5 Conclusion

In this study, the response of tea plants, bacteria, and fungi under 5-year N fertiliser application conditions were systematically investigated. Excessive N fertiliser application decreased tea yield and quality. Moreover, N fertiliser application significantly decreased bacterial and fungal diversity and altered bacterial and fungal compositions and communities. N fertiliser application favoured the growth of copiotrophic taxa (Proteobacteria, Actinobacteria, and Ascomycota) and inhibited oligotrophic taxa (Acidobacteria and Basidiomycota). Bacteria are more sensitive to environmental variations than fungi, and soil pH was the most important factor driving bacterial and fungal community succession. Stochastic processes contributed more to bacterial community assembly than to fungal community assembly in tea plantation soil. Tea yield and quality were more associated with fungi than bacteria based on random forest analysis. In conclusion, considering the extreme environment of tea plantation soil (i.e., low pH and high F and Al contents) and the tolerance of fungi for extreme environments, future studies should investigate the association between fungi and tea growth.

Declarations

Author contributions

Sheng Tang: Conceptualisation, Methodology, Formal analysis, Writing - Original Draft. **Lianghuan Wu:** Conceptualisation, Methodology, Formal analysis. **Qingxu Ma:** Conceptualisation, Methodology, Formal analysis, Writing - Original Draft. **Tong Qi:** Conceptualisation, Methodology, Formal analysis, Writing - Original Draft. **Zhengbo Ma:** Investigation. **Rui Tang:** Investigation. **Wankun Pan:** Investigation. **Haoran Fu:** Investigation. **Jingjie Zhou:** Investigation. **MengXu:** Investigation.

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Figures

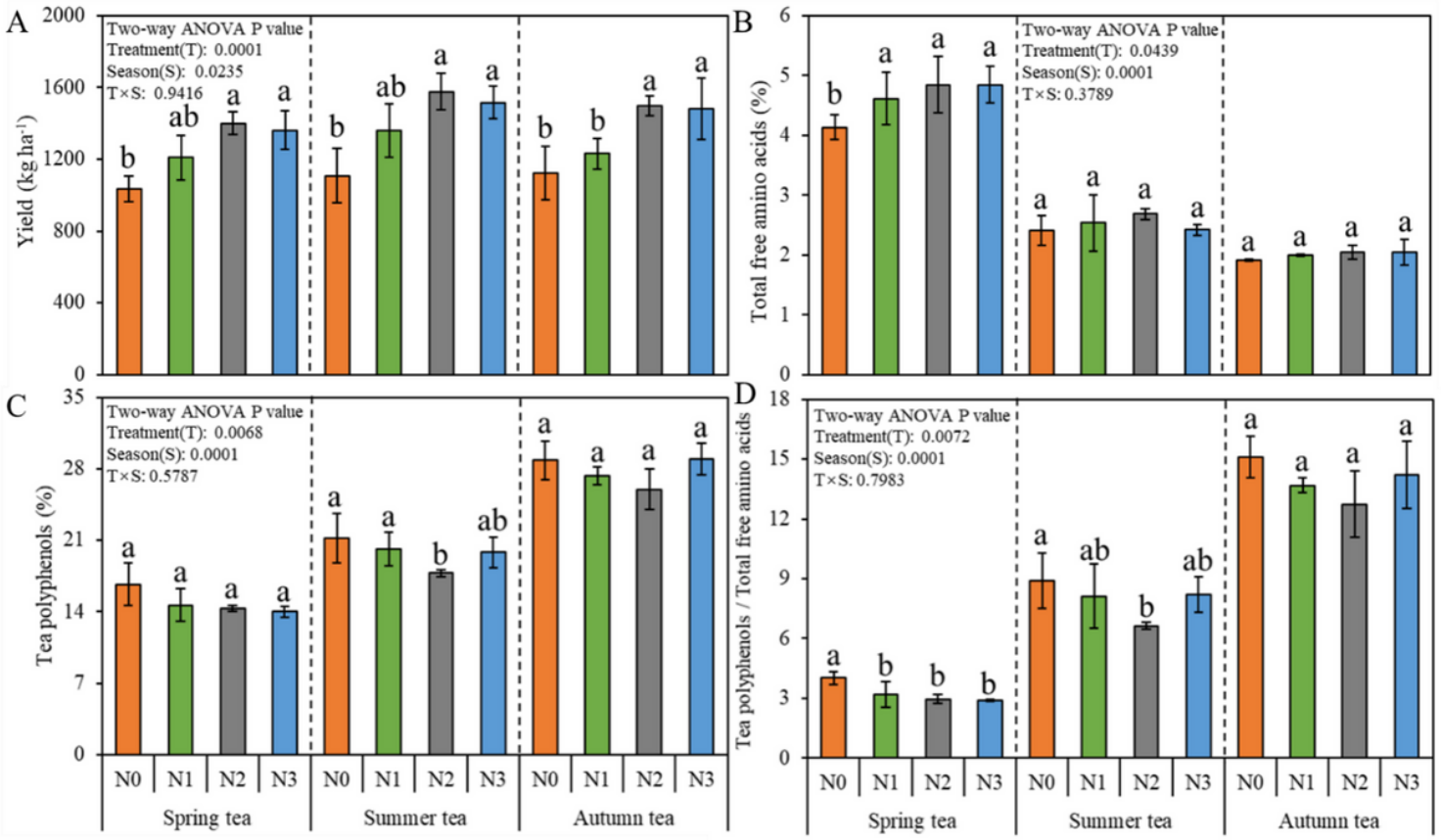

Figure 1

Tea yield and quality under different N fertiliser rates in spring, summer, and autumn. Standard deviations of three replicates are represented as error bars. Different letters above the bars indicate significant differences ($P < 0.05$) between N fertiliser treatments in the same harvest season. N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha−1 y−1 for 5 years.

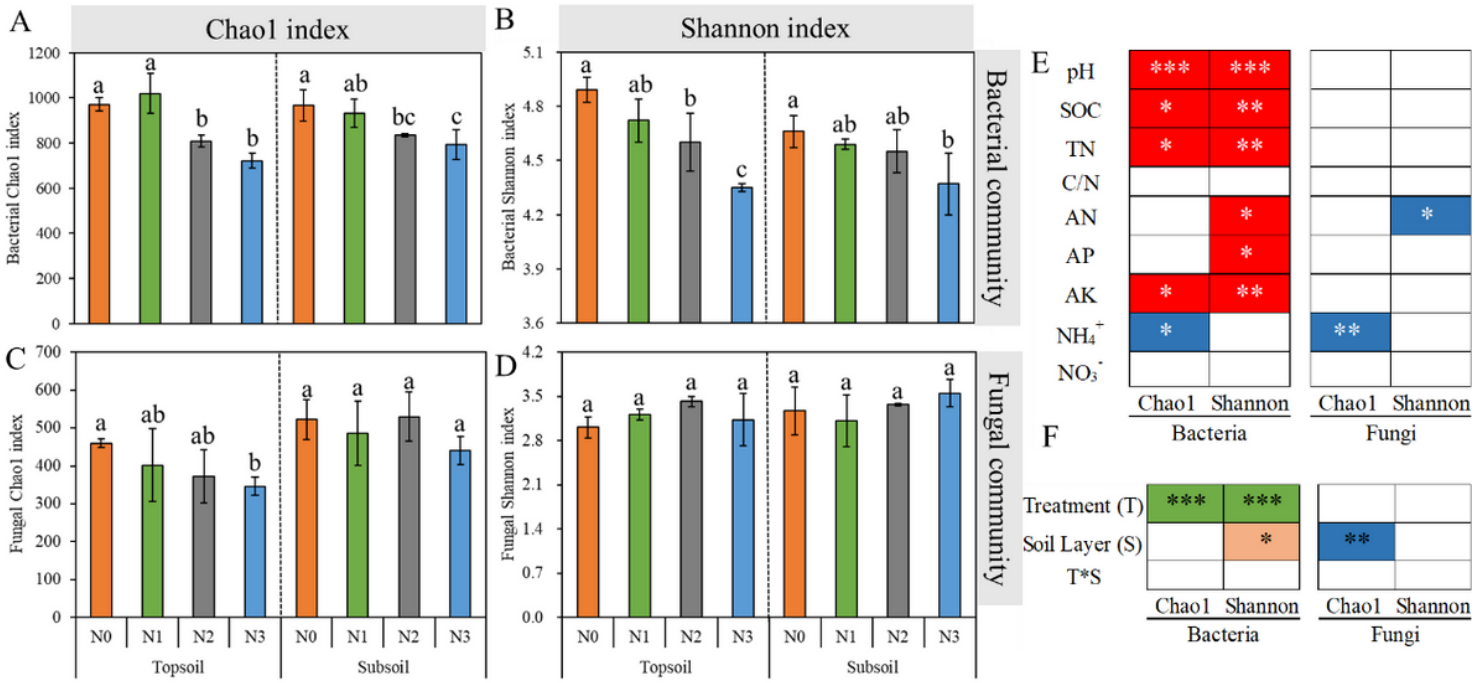

Figure 2

Chao1 and Shannon indices of (A, B) bacterial and (C, D) fungal communities under different N fertiliser application rates. Standard deviations of three replicates are represented as error bars. Different letters above the bars indicate significant differences (P < 0.05) between N fertiliser treatments under the same soil layer (topsoil: 0–25 cm, subsoil: 25–50 cm). N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha−1 y−1, respectively, for 5 years. (E) Pearson's correlation between soil properties and alpha diversity of bacteria and fungi. Red and blue boxes indicate positive and negative correlations between alpha diversity index and soil properties. (F) Two-way ANOVA analysis results; *P < 0.05, **P < 0.01, and ***P < 0.001. SOC: soil organic carbon; TN: total nitrogen; AN: alkali-hydrolysable N; AP: available phosphate; AK: available potassium.

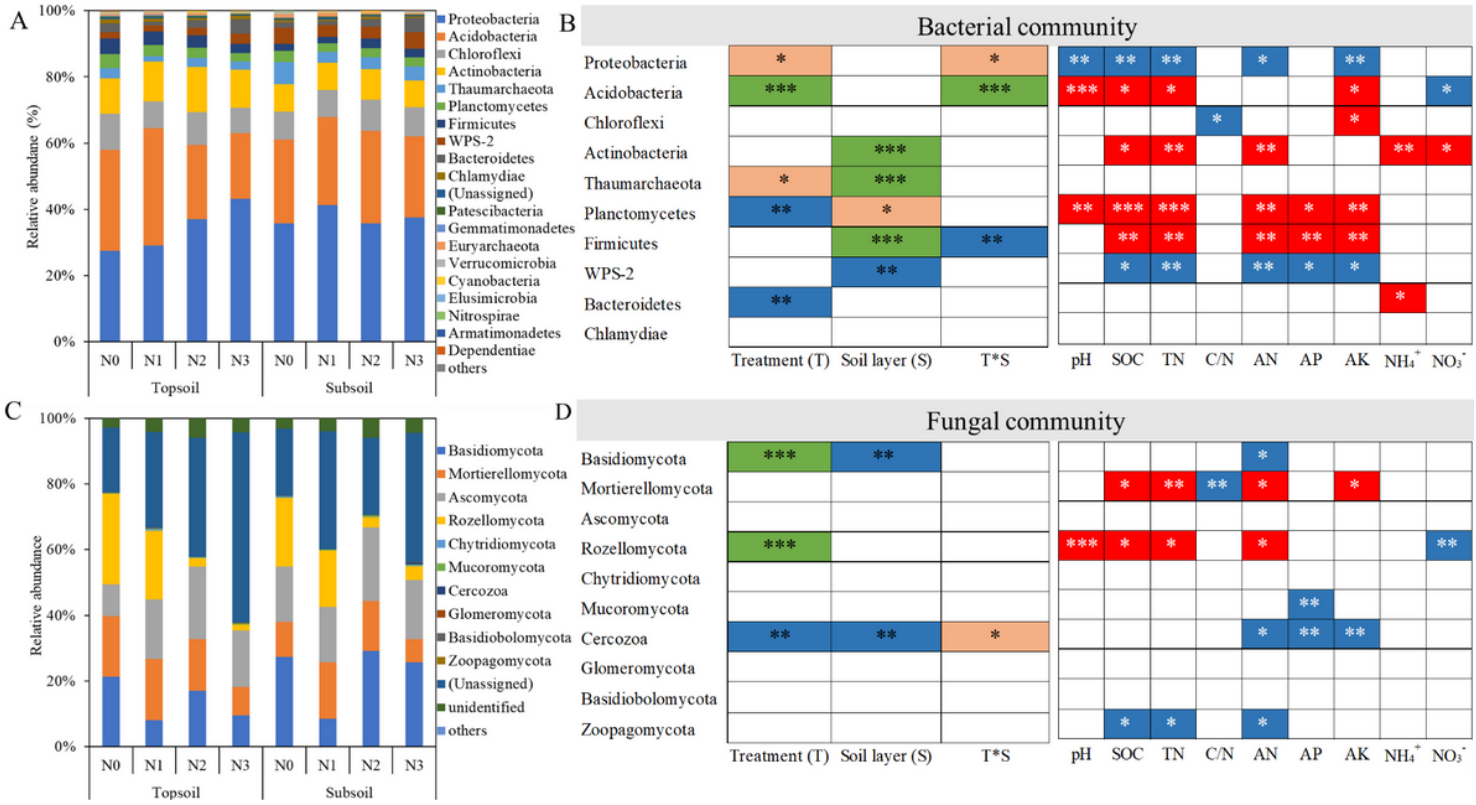

Figure 3

Major phyla of (A) bacterial and (C) fungal communities under different N fertiliser application rates and soil layers (topsoil: 0–25 cm, subsoil: 25–50 cm). Results from two-way ANOVA and Pearson's correlation analysis between soil properties and relative abundance of top 10 (B) bacterial and (D) fungal phyla. *P < 0.05, **P < 0.01, and ***P < 0.001. Red and blue boxes indicate positive and negative correlations between the relative abundance of major phyla and soil properties, respectively. SOC: soil organic carbon; TN: total nitrogen; AN: alkali-hydrolysable N; AP: available phosphate; AK: available potassium. N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha−1 y−1, respectively, for 5 years.

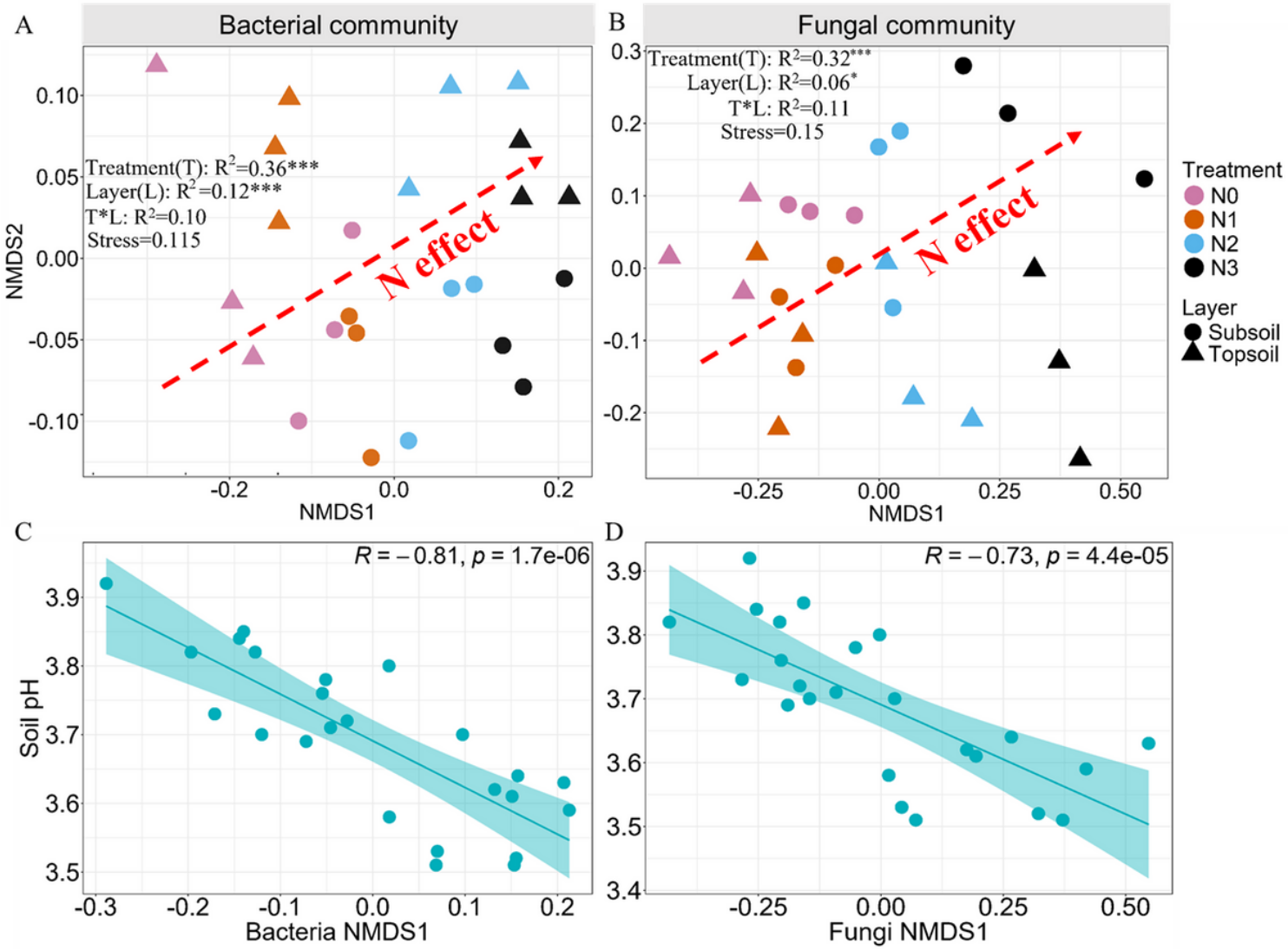

Figure 4

Non-metric multidimensional scaling (NMDS) based on the Bray–Curtis dissimilarities of (A) bacterial and (B) fungal communities in the topsoil (0–25 cm) and subsoil (25–50 cm) under different N application rates. N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha−1 y−1, respectively, for 5 years. Linear regressions for (C) bacterial and (D) fungal NMDS1 values and soil pH values. Shaded areas represent 95% confidence intervals.

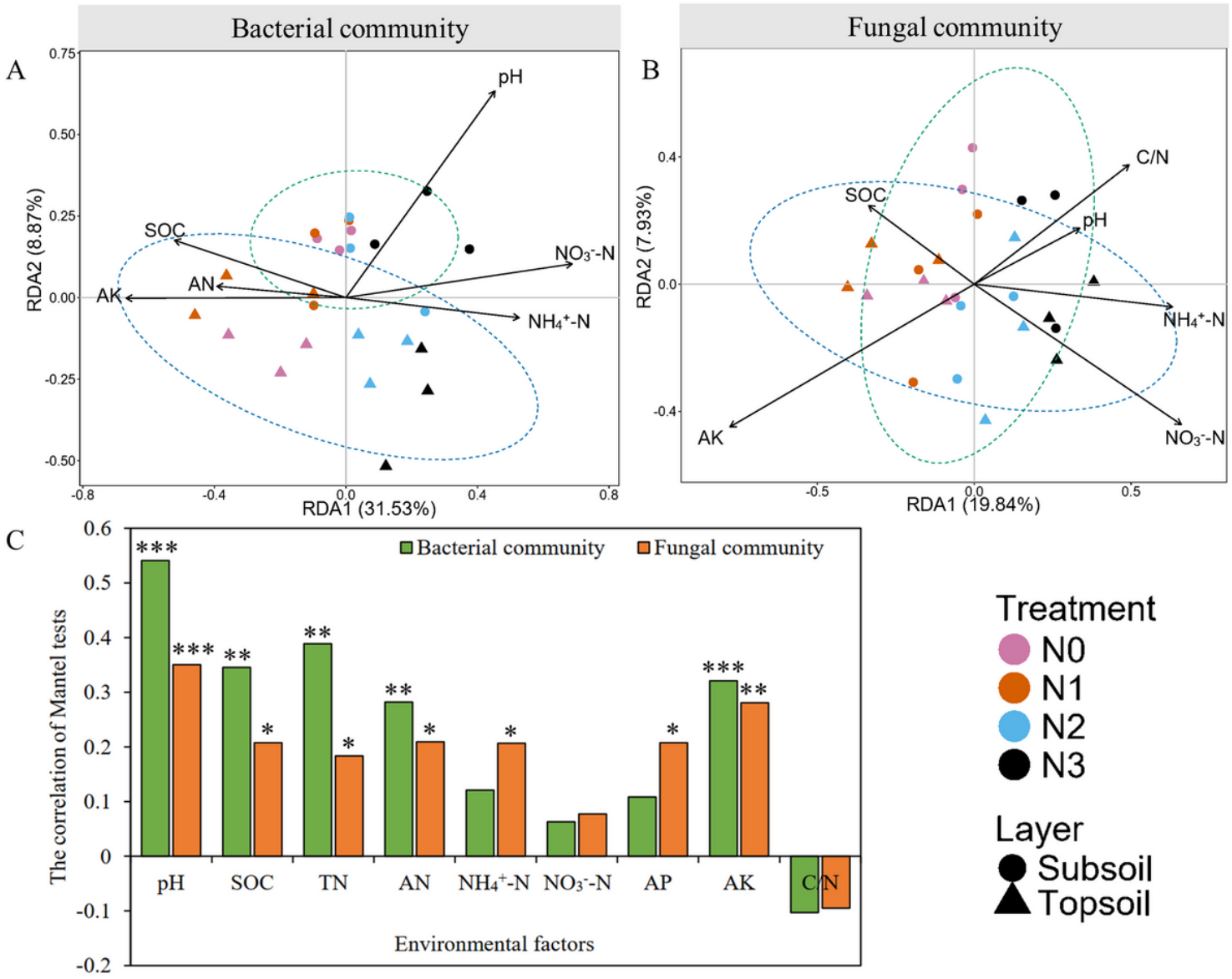

Figure 5

Canonical redundancy analysis (RDA) of (A) bacterial and (B) fungal communities with different N fertiliser application rates and soil layers (topsoil: 0–25 cm, subsoil: 25–50 cm). N0, N1, N2, and N3 refer to N fertiliser application rates of 0, 120, 360, and 600 kg ha−1 y−1, respectively, for 5 years. (C) Mantel's analysis of the relationships between soil bacterial and fungal community structures and environmental variables. SOC: soil organic carbon; TN: total nitrogen; AN: alkali-hydrolysable N; AP: available phosphate; AK: available potassium.

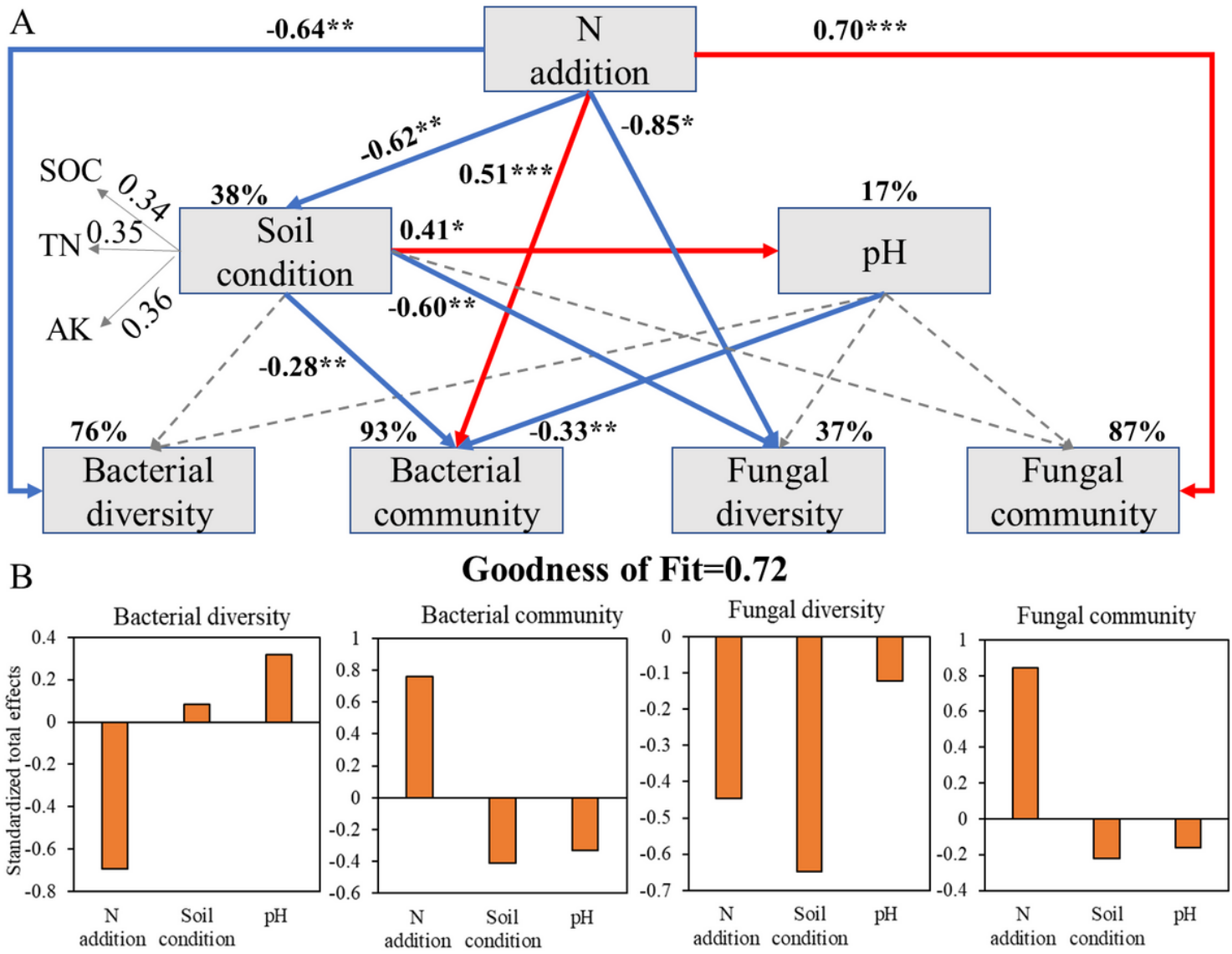

Figure 6

(A) Partial least squares path modelling (PLS-PM) analysis of direct and indirect influences of N fertiliser application on bacteria and fungi. Observed or latent variables are illustrated in the box. 1,000 bootstraps were conducted to estimate path coefficients. Positive and negative effects are presented by red and blue arrows, respectively. Path coefficients that were insignificantly different from zero are shown as dashed lines; *P < 0.05, **P < 0.01, and ***P < 0.001. Percentages above the boxes represent the explanatory degree of variables. The goodness-of-fit was used to assess the model. (B) Standardised total effects of N fertiliser application, soil condition, and soil pH on bacteria and fungi. SOC: soil organic carbon; TN: total nitrogen; AK: available potassium.

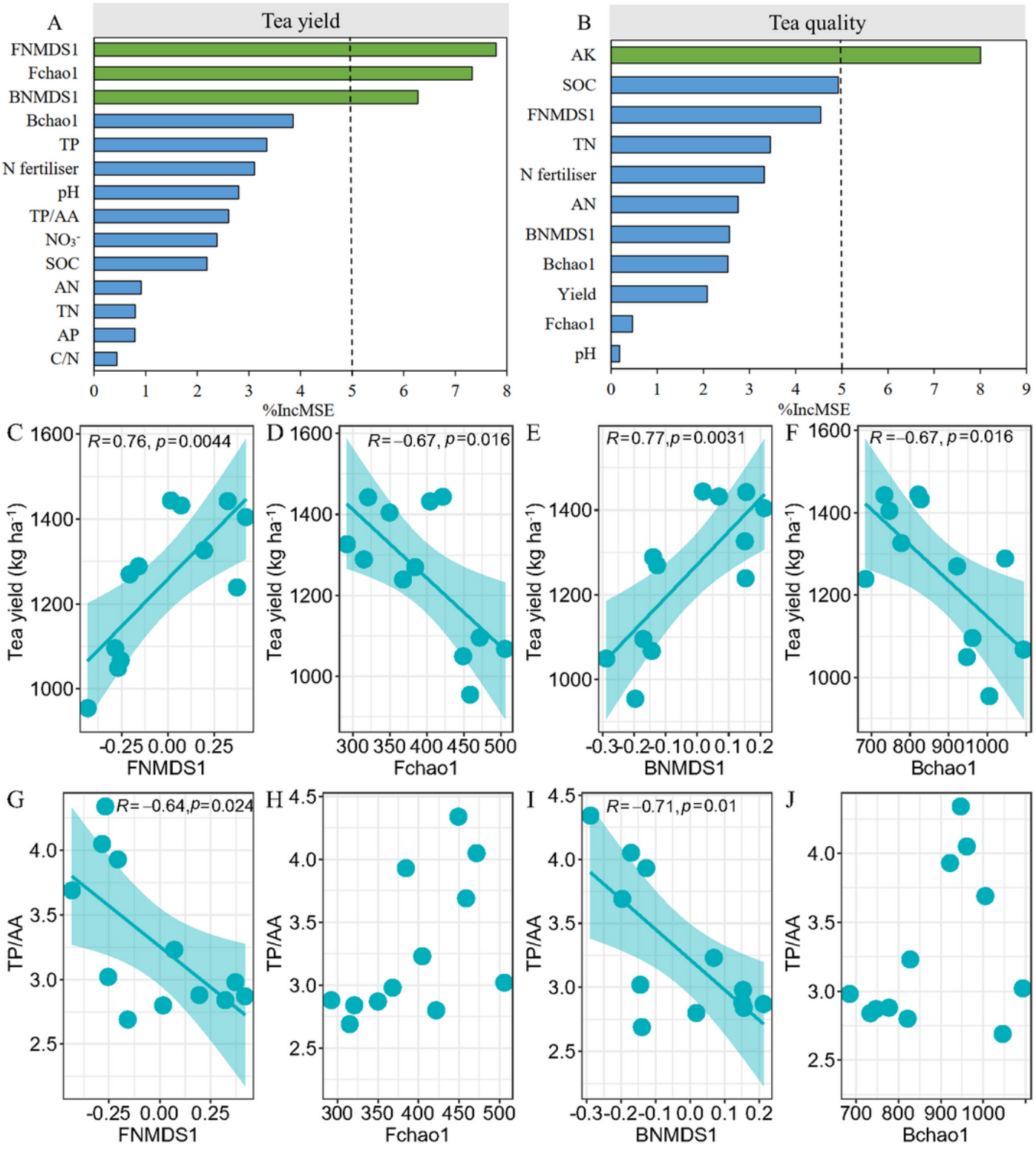

Figure 7

Random forest analysis for the determination of factors affecting tea (A) yield and (B) quality. Pearson's correlation analysis between microbiome and tea (C-J). BNMDS1, FNMDS1: Non-metric multidimensional scaling (NMDS) values based on the Bray–Curtis dissimilarities of bacterial and fungal communities, respectively; Bchao1 and Fchao1 are Chao1 indices of bacterial and fungal communities, respectively; SOC: soil organic carbon; TN: total nitrogen; AN: alkali-hydrolysable N; AP: available phosphate; AK:

available potassium; TP: total polyphenols; TP/AA: ratio of tea polyphenol to total free amino acids; N fertiliser: N fertiliser application rate.

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