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RESEARCH ARTICLE

# Soil microbial networks mediate long-term effects of nitrogen fertilization on ecosystem multiservices

**Yaodan Zhang**[1] | **Ying Wang**[2] | **Guiyao Zhou**[3,4] | **Daniel Revillini**[3] | **Huiying Liu**[5,6] | **Shujuan Wu**[1] | **Ning Chen**[7,8] | **Baoming Du**[9] | **Jingrun Xu**[1] | **Qingwei Li**[1] | **Ding Guo**[1] | **Manuel Delgado-Baquerizo**[3] | **Decao Niu**[1] | **Hua Fu**[1] | **Xiaobo Yuan**[1]

[1]State Key Laboratory of Herbage Improvement and Grassland Agroecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, Engineering Research Center of Grassland Industry, Ministry of Education, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China; [2]Linze Desert Ecosystem Research Station, Gansu Desert Control Research Institute, Lanzhou, China; [3]Laboratorio de Biodiversidad y Funcionamiento Ecosistémico, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Sevilla, Spain; [4]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany; [5]Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, Center for Global Change and Ecological Forecasting, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China; [6]Institute of Eco-Chongming (IEC), East China Normal University, Shanghai, China; [7]State Key Laboratory of Herbage Improvement and Grassland Agro-Ecosystems, College of Ecology, Lanzhou University, Lanzhou, China; [8]Yuzhong Mountain Ecosystem Observation and Research Station, Lanzhou University, Lanzhou, China and [9]School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

**Correspondence**
Manuel Delgado-Baquerizo
Email: m.delgadobaquerizo@gmail.com

Xiaobo Yuan
Email: yuanxb@lzu.edu.cn

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## Abstract

1. Nitrogen (N) fertilization is known to impact the capacity of ecosystems to support multiple ecosystem services such as carbon sequestration and nutrient cycling, particularly in nutrient-limited environments. Yet, little is known about how N fertilization may result in trade-offs across contrasting soil ecosystem services. Moreover, the contribution of soil microbial networks as mediators of the impacts of fertilization on soil ecosystem services is poorly understood.
2. Here we collected topsoil (0–10 cm) and subsoil (10–20 cm) samples from a 13-year N addition experiment in a semiarid grassland to investigate how long-term N additions affect soil multiservices.
3. We found that soil multiservice predominantly exhibited a hump-shaped response to the increasing levels of N addition across two soil depths. More importantly, changes in the complexity of soil microbial networks were positively correlated with ecosystem multiservices across the two soil depths. This relationship was especially important in explaining topsoil multiservice responses, while in subsoils, multiservices were more strongly associated with abiotic properties than network complexity. This distinction may be attributed to the lower microbial activity and reduced nutrient utilization capacity in subsoils, which allows abiotic factors to play a more dominant role on multiservices.

4. *Synthesis*. Our results highlight that soil microbial network complexity is highly correlated with multiple ecosystem services in the context of global atmospheric N deposition.

**KEYWORDS**
ecological cluster, ecosystem services, microbial co-occurrence network, mineral protection, network complexity, nitrogen deposition and input, soil function

## 1 | INTRODUCTION

Nitrogen (N) fertilization caused by anthropogenic activities such as fertilizer application and fossil fuel combustion has significantly impacted the capacity of ecosystems to support key services such as nutrient cycling and soil carbon (C) sequestration (Pichon et al., 2024; Yang et al., 2024). Yet, the mechanisms behind such impacts are still unclear. The impact of N fertilization is especially noticeable in drylands, which cover almost half of the planet and support the well-being of millions of people around the world (Maestre et al., 2012; Püspök et al., 2023). These typically nutrient-limited ecosystems are especially responsive to N additions with consequences for the maintenance of system-wide biodiversity and functioning (Delgado-Baquerizo et al., 2017; Zhou et al., 2024). In general, low levels of N inputs may facilitate plant productivity and soil nutrient cycling in drylands, enhancing soil microbial diversity and carbon (C) sequestration. Conversely, high levels or long durations of N inputs may disrupt soil nutrient balance, limiting the growth of N-sensitive or less competitive microbes, which may indirectly reduce microbial diversity or directly suppress it through soil acidification and ion toxicity (Liu et al., 2020, 2021). Understanding the long-term effects of N fertilization on drylands is of critical importance for the conservation of these ecosystems and the many services they provide.

Soil microbes are the most diverse and abundant organisms on Earth (Anthony et al., 2023; Bahram et al., 2018). They are also the major regulators of function in drylands worldwide (Delgado-Baquerizo et al., 2017). Yet how soil microbes modulate the responses of ecosystem multiservices, including soil health, biodiversity conservation, as well as nutrient cycling, to fertilization is still poorly understood. N fertilization may indirectly affect ecosystem multiservices by changing soil microbial diversity and altering soil physicochemical properties. In addition, it is worth noting that soil microbial diversity encompasses not only species richness and abundance but also the complex interactions such as predation, competition, mutualism, parasitism and amensalism among the hyper-diverse microorganisms below-ground (Faust & Raes, 2012; Liu et al., 2025). These interactions can be represented by microbial association networks, that is, complex networks composed of linkages or interactions formed between all identified microbial taxa (Delgado-Baquerizo et al., 2020; Guseva et al., 2022; Yuan et al., 2021). In these co-occurrence networks, soil microbial taxa experiencing similar environmental and edaphic filtering tend to cluster together, forming ecological clusters with relatively strong and persistent associations (Delgado-Baquerizo et al., 2020). More importantly, soil microbial ecological clusters and the complexity of co-occurrence networks reflect the dynamics of microbial community structure and interpopulation relationships. These features have been identified as critical regulators of ecosystem function, often surpassing microbial diversity itself in importance (Wang, Zhang, et al., 2023; Yuan et al., 2021; Zhang et al., 2025). Much less is known about the contribution of soil microbial network complexity and its ecological clusters in natural ecosystems in explaining the response of soil multiservices (SMS) to long-term atmospheric N deposition, especially in the context of highly vulnerable drylands. While there have been strides made to explore SMS across many ecosystems and under different environmental conditions (Rillig et al., 2023; Scherzinger et al., 2024; Zhou et al., 2022), the ecosystem functions and services provided and supported by subsoils are often overlooked. This is despite the fact that subsoils are critical components of terrestrial ecosystems, storing about half of the soil C, N and phosphorus (P) and hosting a large and diverse microbial community (Harrison et al., 2011; He et al., 2022). In addition, plant C input, soil physicochemical properties and microbial properties in the topsoil differ significantly from those in the subsoil in grassland ecosystems (Wang, Li, et al., 2023). This distinction may result in drastically different multiservices provided by topsoil or subsoil; however, systematic studies investigating the effects of N addition on soil multiservices and the extent to which regulatory mechanisms vary with soil depth remain insufficient.

To address this knowledge gap, we conducted a 13-year N addition experiment with six N addition levels in a semiarid grassland from China to explore how soil microbial diversity, network complexity and ecological clusters (determined using metagenomics-based taxonomic information) affect SMS composed of 17 soil functions associated with six ecosystem services including soil carbon stocks, nutrient supply, biodiversity, soil organic matter decomposition, pathogen control and plant–soil symbiosis. These investigations were carried out in both the topsoil (0–10 cm) and subsoil (10–20 cm) layers. We expected ecosystem services to follow a hump-shaped pattern in response to N fertilization, with the highest levels observed at intermediate N addition levels (Wu et al., 2023; Xing et al., 2022; Yang et al., 2023). This expectation is based on the intermediate disturbance hypothesis that suggests that a bit of disturbance (i.e. low-moderate N addition relative to local conditions) can have a positive effect on the ecosystem functional capacities, but too much disturbance (i.e. relatively high N addition) can result in the collapse of ecosystem functions and services. We also expected N addition effects on multiservices to be indirectly regulated by soil microbial network complexity (Long et al., 2025;

Manning et al., 2018). Since microbial resource availability decreases with increasing soil depth, this regulatory mechanism may also vary with soil depth (Chen et al., 2021; Wang, Li, et al., 2023). This study will provide important insights into the role of dryland microbial networks in mediating the effects of N enrichment, which might serve as an approachable indicator for soil functional capacity across this globally critical ecosystem.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description and experimental design

The study was conducted at the Semiarid Climate and Environment Observation Station of Lanzhou University (SACOL), located in Gansu Province, northwest China (35°57′ N, 104°09′ E; 1966 m a.s.l). The climate of this area belongs to continental semiarid, with a mean annual temperature of 6.7°C and a mean annual precipitation of 382 mm based on the past 50 years meteorological data (Yuan et al., 2019). Most of the precipitation occurs from July to September. According to the FAO/ISRIC/ISSS soil classification system (FAO/ISRIC/ISSS, 1998), the soil is classified as sierozem. The dominant plant species was the perennial bunchgrass *Stipa bungeana* in this semi-arid grassland (Niu et al., 2018; Zhang et al., 2025).

We conducted a N addition experiment in a grassland that was originally fenced off to exclude large vertebrate herbivores in 2004 (Yuan et al., 2019). Urea [$CO(NH_2)_2$] is commonly applied as N fertilizer due to its high N content and good solubility (Pradana et al., 2021). Briefly, 30 4 m × 5 m plots were established following a completely randomized design in 2009 and each plot was separated by at least 0.5 m walkways. This experiment had six treatments with five replicates each, receiving urea at the rate of 0, 1.15, 2.30, 4.60, 9.20 and 13.80 g N m$^{-2}$ year$^{-1}$. Hereafter, treatments will be referred as $N_0$, $N_{1.15}$, $N_{2.30}$, $N_{4.60}$, $N_{9.20}$ and $N_{13.80}$. Among them, $N_{1.15}$ and $N_{2.30}$ are consistent with the rates of current atmospheric N deposition in this site, which was estimated as 0.20–2.2 g N m$^{-2}$ year$^{-1}$ (Lü & Tian, 2007). Higher N levels ($N_{4.60}$ to $N_{13.80}$) were selected to simulate the impact of future atmospheric N deposition on ecosystem structure and function (Yu et al., 2019). Fertilizer was dissolved in 10 L of purified water and uniformly applied to the plot using a sprayer on a rainy day at the end of May and June of each year. Meanwhile, the control plots received the same amount of purified water without fertilizer to eliminate errors caused by soil moisture changes. A detailed description of the experimental set-up can be found in previous studies conducted at this site (Niu et al., 2018; Yuan et al., 2019).

### 2.2 | Plant and soil sampling

To assess the effect of N addition on plant properties, we investigated species richness and the abundance of individual species, and collected plant above-ground living biomass, litter biomass and below-ground biomass in randomly arranged 1 m × 1 m vegetation quadrats in mid-August 2021. Above-ground biomass was collected by clipping it at ground level, after which plant litter was also sampled. Meanwhile, two soil cores (12.5 cm inner diameter) at soil depths of 0–10 cm (topsoil) and 10–20 cm (subsoil) were sampled within each quadrat to measure below-ground plant biomass. Moreover, five soil cores (7.5 cm inner diameter) in the topsoil and subsoil were randomly collected within each quadrat. The soil samples were sieved through sterilized 2 mm mesh to remove roots and rocks; then, the five soil cores were homogeneously mixed into one composite sample and immediately stored in a portable refrigerator for transport to the laboratory. We further divided soil composite samples into three subsamples and stored them at room temperature (soil total nutrients, available P and mineral properties), 4°C (for the determination of soil water holding capacity, clay+silt, inorganic N, microbial respiration and N and P mineralization rates) and −70°C (for soil microbial metagenome sequencing analysis), respectively. All plant biomass samples were oven-dried at 70°C for 48 h to measure their weight (g m$^{-2}$).

### 2.3 | Measurements of soil physicochemical properties and mineral protection

Fresh soil was dried for 24 h at 105°C to measure soil moisture (%). The method described by Bao (2000) was used to determine soil water holding capacity (WHC, %). Soil pH was measured using a suspension sample (soil: water 1:2.5) with a pH meter (PHS-3C, Shanghai, China). Soil samples were passed through a 53 μm mesh to determine the proportions of clay and silt (Qiu et al., 2021). Considering the pivotal role of soil mineral protection in soil C and nutrient cycling, we measured the contents of exchangeable calcium ($Ca_{exe}$) and magnesium ($Mg_{exe}$), and Fe/Al oxides. Among them, $Ca_{exe}$ and $Mg_{exe}$ were extracted with an equal volume of triethanolamine (pH = 8.1) and 1 M $BaCl_2$ (Dohrmann, 2006; Zhang et al., 2025) and then analysed by flame atomic absorption spectrometry (M6AA system, Thermo Scientific, West Palm Beach, FL, USA). In addition, poorly crystalline iron and aluminium oxide ($Fe_o + Al_o$) and the complexed iron and aluminium oxides ($Fe_p + Al_p$) were extracted with 0.2 M oxalic acid-ammonium oxalate solution and 0.2 M sodium pyrophosphate solution, respectively (Feng et al., 2022; Keiluweit et al., 2015; Zhang et al., 2025). The extracted samples were then analysed with an inductively coupled plasma-optical emission spectrometer (ICAP 6300, Thermo Science, MA, USA).

### 2.4 | Soil shotgun metagenomics sequencing

#### 2.4.1 | DNA extraction, metabarcoding and metagenomics library preparation

We used the FastDNA® SPIN Kit for Soil (MP Biomedicals, OH, USA) to extract the metagenomic DNA from each soil sample following the manufacturer's instructions. The DNA concentration, integrity and purity were assessed with the Agilent Fragment Analyzer 5400 (Fragment Analyzer 5400, Agilent Technologies Co., Ltd., CA, USA). The DNA samples were then randomly fragmented into ~350 bp pieces using a Covaris ultrasonic fragmentation system (Covaris M220, Covaris S2 System, MA, USA). Libraries were constructed using the NEB Next®Ultra™ DNA Library Prep Kit for Illumina (NEB, MA, USA), with index codes incorporated into the attribute sequences of each sample. Library preparation involved end repair, addition of polyA tails, sequencing connectors, purification and PCR amplification. Finally, index-coded samples were clustered on the cBot Cluster Generation System using the Illumina PE Cluster Kit (Illumina, CA, USA) as per the manufacturer's protocol. Following cluster generation, the DNA library was sequenced on the Illumina NovaSeq 6000 platform, producing 150 bp paired-end reads (Zhang et al., 2025).

### 2.4.2 | Bioinformatics analysis

Shotgun metagenomics sequencing was conducted on the Illumina NovaSeq high-throughput sequencing platform to obtain raw metagenomics data (raw data) of soil microbes in each sample (Zhang et al., 2025). To ensure data reliability, the raw sequencing data were pre-processed using Kneaddata software with the following steps: Firstly, utilizing Trimmomatic, joint sequences (parameter: ILLUMINACLIP: adapters_path:2:30:10) were removed, sequences of low quality (default quality score threshold ≤20; parameter: SLIDINGWINDOW:4:20) and shorter than 50 bp (parameter: MINLEN:50) were filtered out. Secondly, to address potential host contamination, the clean data were aligned with the host genome using Bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml, parameter: --very-sensitive) to filter host sequences and obtain valid data for further analysis. Thirdly, the quality control steps were validated using FastQC (Langmead & Salzberg, 2012). Finally, Kraken2 was employed to calculate the number of sequences of the species in the soil samples by comparing them with a self-constructed microbial nucleic acid database. The relative abundance of species was predicted using Bracken. Kraken2, a modern K-mer-based comparison tool, utilized a local database containing 16,799 known bacterial genomes (Lu et al., 2017).

## 2.5 | Soil microbial co-occurrence networks analyses

The microbial co-occurrence network was constructed based on the abundance of operational taxonomic units (97% OTUs) of microbial taxa (fungi, bacteria and archaea) to assess the effects of N addition and soil depth on soil microbial interactions. Four core ecological clusters in the topsoil and subsoil were identified using the Fruchterman-Reingold algorithm from the *igraph* package in R (Csardi, 2013). OTUs with Spearman correlation coefficients >0.65 and $p < 0.05$ were retained in the construction of the soil microbial co-occurrence network (Jiao, Lu, et al., 2022). This data screening approach ensures that OTUs with possible interactions can be identified, which minimizes potential for spurious correlations. Microbial co-occurrence networks and their topological characteristics were visualized by *igraph* software to evaluate the complexity of the microbial network (Newman, 2003). The topological characteristics of the networks include the number of nodes and edges, average path length, average degree and transitivity. Among them, average path length indicates the average distance between all pairs of nodes; average degree represents the average number of connections each unique target node has with every other node; transitivity measures the tightness degree of the interconnectedness between nodes. We standardized network topological characteristics (nodes, edges and average path length) based on the (raw-min(raw)/(max(raw)-min(raw))) calculation method, and their mean values were used to characterize microbial co-occurrence network complexity (Yao et al., 2024).

## 2.6 | Determination of individual soil variables

We measured 17 variables related to six soil services, including (1) C stocks: soil organic C (SOC); (2) nutrient supply: total N (TN), total phosphorus (TP), ammonium-N ($NH_4^+$-N), nitrate-N ($NO_3^-$-N), available inorganic phosphorus (SAP); (3) biodiversity: fungal, bacterial and archaeal richness; (4) soil organic matter (SOM) decomposition: the rate of N mineralization (NMR), the rate of P mineralization (PMR), soil microbial respiration (SMR), C-acquiring enzyme (β-1,4-glucosidase, BG), N-acquiring enzymes (β-1,4-N-acetylamino glucosidase, NAG; leucine aminopeptidase, LAP), P-acquiring enzyme (alkaline phosphatase, AP); (5) pathogen control; and (6) plant–soil symbiosis (Figure S1). The variables such as these constitute a good proxy for multiple ecosystem services associated with soil nutrient pool accumulation, organic matter decomposition and microbial diversity (Scherzinger et al., 2024; Zhou et al., 2022). See Table S1 for further rationale for selection of the variables. SOC and TN were measured by a CHN elemental analyzer (Flash EA1112, Thermo Scientific, West Palm Beach, FL, USA). The nitric acid-microwave digestion, followed by a molybdate colorimetric test, was used to determine TP. The concentrations of $NH_4^+$-N and $NO_3^-$-N were measured using 2 M KCl extracts based on the method of vanadium reduction and the Griess assay. SAP concentration was analysed following a 0.5 M $NaHCO_3$ extraction. The activities of BG, NAG, LAP and AP were measured in black polystyrene 96-well, 300-ml microplates fluorometrically using 4-methylumbelliferyl (MUB) substrates, following the method described by DeForest (2009). We used an aerobic incubation procedure to determine SMR, NMR and PMR based on the protocol proposed by Wang, Niu, et al. (2023), and details were provided in the Supporting Information: Methods. Based on species-level fungal abundance in soil samples obtained through metagenomic sequencing, the FUNGuild database was utilized to determine the identity of soil-borne fungal potential plant pathogens and arbuscular and ectomycorrhizal fungi, as well as to calculate their relative abundances. Only highly probable and

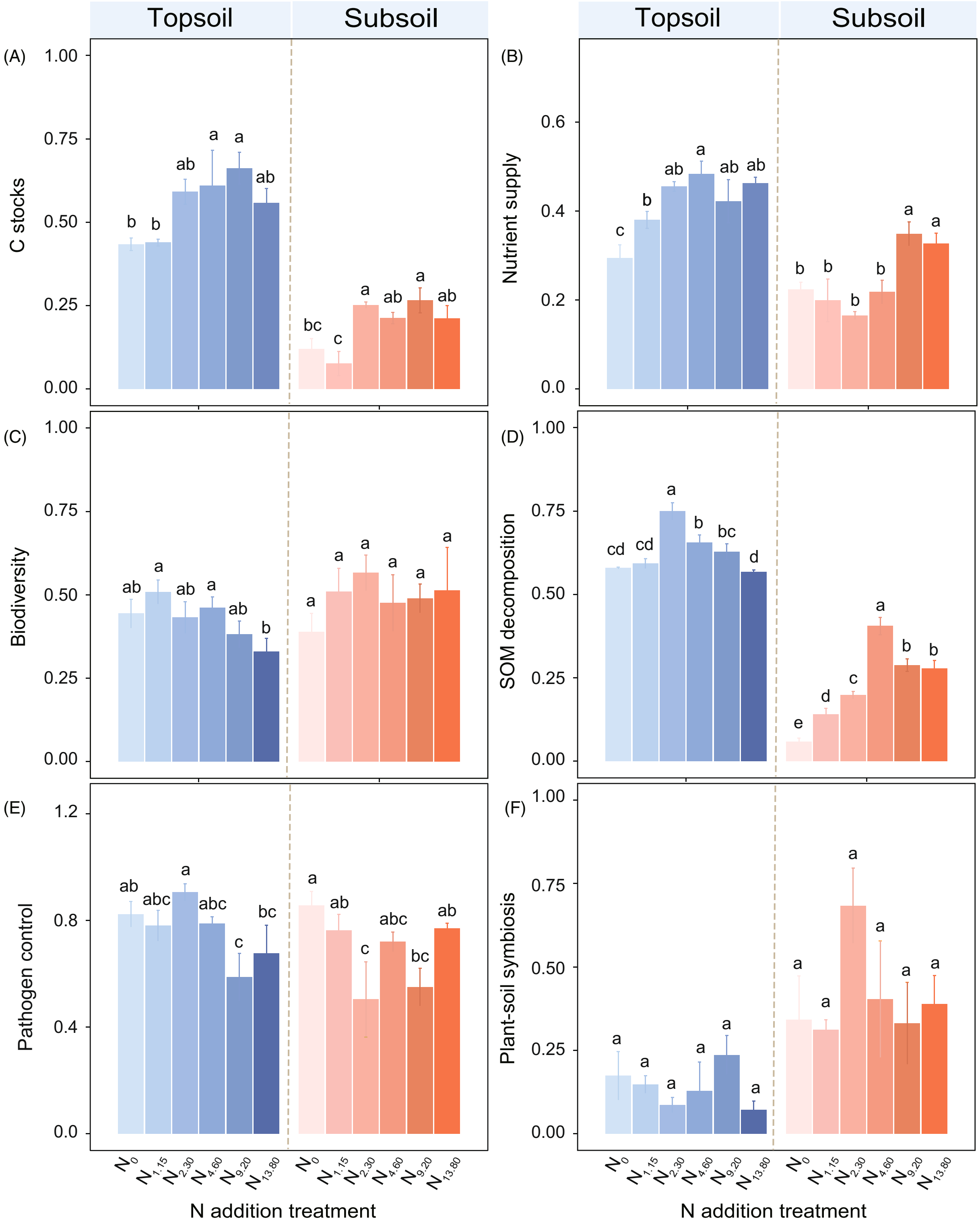


**FIGURE 1** Effects of N addition on soil C stocks (A), nutrient supply (B), biodiversity (C), SOM decomposition (D), pathogen control (E) and plant–soil symbiosis (F) in the topsoil and subsoil. Soil nutrient supply, biodiversity and SOM decomposition were calculated as the average of multiple soil variables. The bars are average values with the error bars denoting standard errors (SE; $n = 5$). Different lowercase letters indicate significant differences among the N addition treatments using *Duncan's* post hoc test ($p < 0.05$). $N_0 = 0\,g\,N\,m^{-2}\,year^{-1}$; $N_{1.15} = 1.15\,g\,N\,m^{-2}\,year^{-1}$; $N_{2.30} = 2.30\,g\,N\,m^{-2}\,year^{-1}$; $N_{4.60} = 4.60\,g\,N\,m^{-2}\,year^{-1}$; $N_{9.20} = 9.20\,g\,N\,m^{-2}\,year^{-1}$; $N_{13.80} = 13.80\,g\,N\,m^{-2}\,year^{-1}$.

probable guilds were used in our analysis. Pathogen control was the inverse of the proportion of plant pathogens, and plant–soil symbiosis was the proportion of arbuscular and ectomycorrhizal fungi (Rillig et al., 2023; Zhou et al., 2023).

## 2.7 | Soil multiservices measures

Soil multiservices (SMS) is a quantitative index which provides an easily interpretable and straightforward assessment of the ability of ecosystems to support multiple services simultaneously (Manning et al., 2018). In this study, three complementary approaches including the averaging method, the multiple threshold method, as well as the service dimensions approach were used to calculate soil multiservices.

(i) The averaging method assesses changes in multiple soil services simultaneously by integrating multiple soil services into an individual index, and it is the most adopted method for measuring soil multiservices. In this study, all individual soil services were first normalized, and then the treated soil services were averaged to calculate the soil average multiservices ($SMS_a$). Although the average method visualizes the average level of ecosystem service, it ignores the trade-offs and synergies between services (Vandandorj et al., 2017; Zhou et al., 2022). In order to overcome the potential shortcomings of the average SMS method and obtain the number of services that perform well in the presence of the corrections among services, we also adopted the multiple-threshold approach to calculate soil multiservices, and details are provided in the Supporting Information: Methods.

(ii) The ecological service network was employed to assess different multiservice dimensions, where individual soil services in each dimension were highly correlated (Spearman correlation coefficients >0.0 and $p<0.05$). At the same time, we could intuitively understand the relative importance of different soil services of each dimension (i.e. the size of nodes) in the ecological service network (Fan et al., 2023). The multiservices of each ecological dimension were calculated by averaging the standardized soil services. Spearman correlation coefficients >0.2 and $p<0.05$ were used to construct a soil ecological service network and visualized using the Gephi platform (https://gephi.org/).

## 2.8 | Statistical analyses

All variables were assessed for normality and homogeneity before testing for significance in differences, and data were $\log_{10}$-transformed when necessary. One-way analysis of variance (ANOVA) with *Duncan's* post hoc test was conducted to compare the effect of N addition on plant properties, soil physicochemical properties, mineral protection, microbial α-diversity, microbial network complexity, soil services and soil multiservices at each soil depth. Meanwhile, two-way ANOVA was performed, using the soil depth as a between-subject effect and N addition treatment as the within-subject effect, to analyse the responses of these variables to N addition, soil depth and their interactions.

We used a polynomial non-linear regression analysis to fit how soil multiservices and the topological features of the microbial network change with increasing levels of N addition. The ordinary least squares linear regression analysis was conducted to investigate the relationships between soil microbial diversity and network complexity and multiservices. The correlations of the microbial network complexity and ecological cluster abundance with plant properties, soil physicochemical properties, soil mineral protection and soil multiservices were assessed using the *Spearman* function of the *Heatmap* package (Kolde & Kolde, 2015). Boosted regression tree (BRT) analysis was employed to evaluate the relative contributions of plant properties, soil physicochemical properties, mineral protection, microbial network complexity and ecological cluster abundance to soil multiservices.

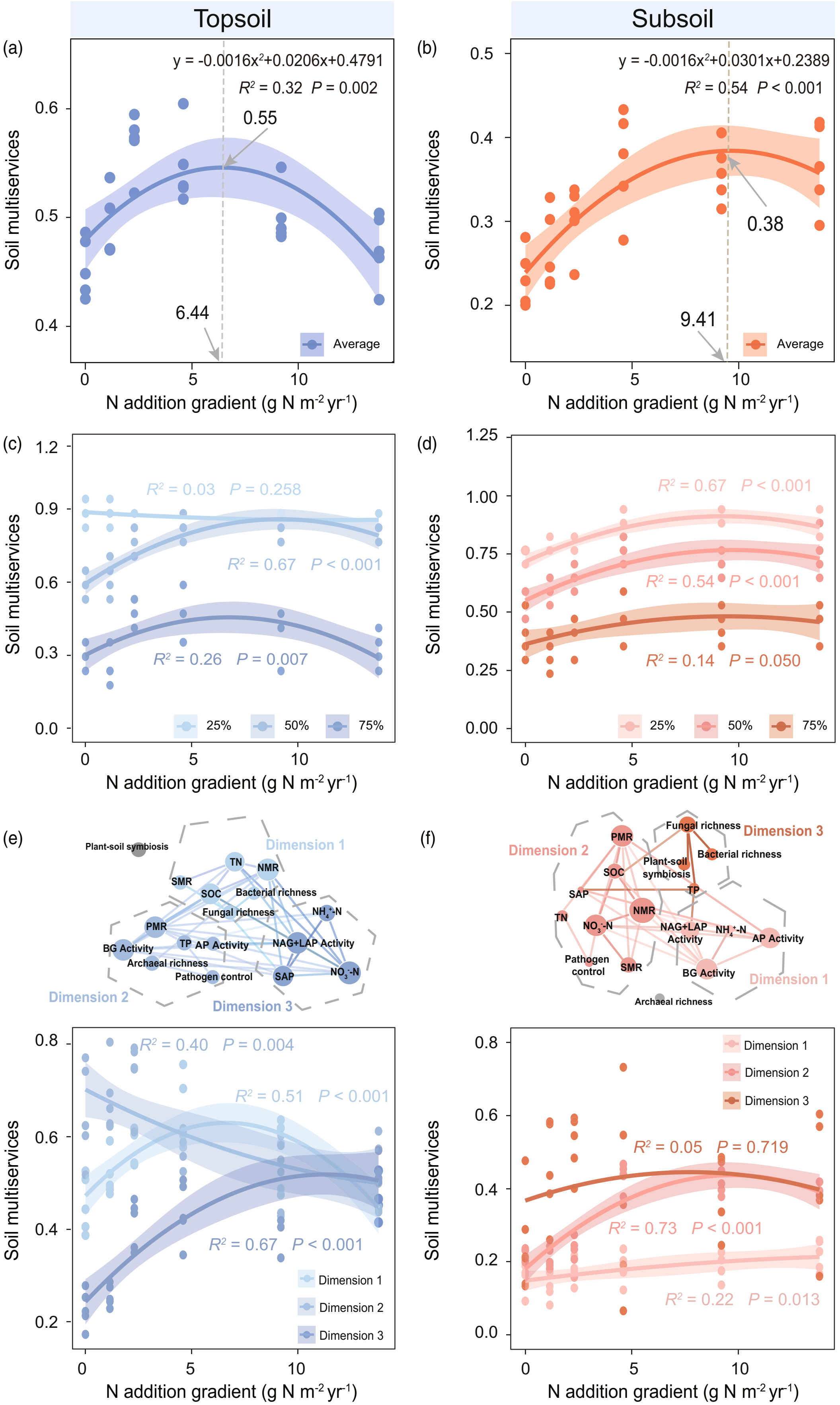
Piecewise structural equation modelling (*piecewise* SEM) was conducted to evaluate the direct and indirect regulatory paths of plant properties, soil physicochemical properties, soil mineral protection, microbial network complexity and ecological cluster abundance on soil multiservices under N addition for the two soil depths (Lefcheck, 2016). Details on model construction and evaluation were presented in Supporting Information: Methods. All statistical analyses were conducted using R version 4.1.2 (R Development Core Team, 2023), with a statistical significance threshold set at $p=0.05$. All data were generated and visualized by the *ggplot2* package in R (Wickham, 2011).

# 3 | RESULTS

## 3.1 | Effects of N addition on soil multiservices

N fertilization impacted both individual and multiple soil ecosystem services (Figures 1 and 2 and Figure S1). Specifically, C stocks and nutrient supply in the topsoil and subsoil significantly increased with N addition (Figure 1A,B), while fungal pathogen control significantly decreased along the N addition gradient (Figure 1E). SOM decomposition in the topsoil and subsoil showed a hump-shaped response to N addition, with peak values at $N_{2.30}$ and $N_{4.60}$, respectively (Figure 1D). Surprisingly, soil biodiversity was not affected by N

FIGURE 2 Effects of N addition on soil multiservices in the topsoil and subsoil. Soil multiservices include averaged (a, b), different thresholds (c, d) and different dimensions (e, f). In the ecological service networks, nodes represent soil services, with size proportional to their relative importance and highly related soil services are grouped into dimensions. The curves were fitted using non-linear regression models, with shaded areas representing the 95% confidence intervals. $R^2$ and $p$-values are also shown in corresponding dimension colours, respectively. See Figure 1 for treatment abbreviations.


Topsoil
Subsoil
(a)
y = -0.0016x²+0.0206x+0.4791
R² = 0.32 P = 0.002
0.55
6.44
Average
Soil multiservices
0.4
0.5
0.6
0
5
10
N addition gradient (g N m⁻² yr⁻¹)
(b)
y = -0.0016x²+0.0301x+0.2389
R² = 0.54 P < 0.001
0.38
9.41
Average
0.2
0.3
0.4
0.5
(c)
R² = 0.03 P = 0.258
R² = 0.67 P < 0.001
R² = 0.26 P = 0.007
25%
50%
75%
0.0
0.3
0.6
0.9
1.2
(d)
R² = 0.67 P < 0.001
R² = 0.54 P < 0.001
R² = 0.14 P = 0.050
0.00
0.25
0.50
0.75
1.00
1.25
(e)
Plant-soil symbiosis
Dimension 1
TN
NMR
SMR
SOC
Bacterial richness
Fungal richness
NH₄⁺-N
PMR
BG Activity
TP
AP Activity
NAG+LAP Activity
Archaeal richness
Pathogen control
SAP
NO₃⁻-N
Dimension 2
Dimension 3
R² = 0.40 P = 0.004
R² = 0.51 P < 0.001
R² = 0.67 P < 0.001
Dimension 1
Dimension 2
Dimension 3
0.2
0.4
0.6
0.8
(f)
PMR
Fungal richness
Dimension 3
Dimension 2
Bacterial richness
SOC
Plant-soil symbiosis
SAP
TP
NMR
TN
NO₃⁻-N
NAG+LAP Activity
NH₄⁺-N
AP Activity
Pathogen control
SMR
BG Activity
Dimension 1
Archaeal richness
Dimension 1
Dimension 2
Dimension 3
R² = 0.05 P = 0.719
R² = 0.73 P < 0.001
R² = 0.22 P = 0.013
0.0
0.2
0.4
0.6
0.8

addition, soil depth or their interaction, and plant–soil symbiosis was significantly influenced by soil depth, with greater values observed in the subsoil (Table S2).

Based on average, multiple threshold and multiple dimension approaches, we evaluated the response of SMS to N addition (Figure 2). Importantly, we determined that averaged SMS in the topsoil and subsoil presented a hump-shaped response to N addition, though the different soil layers exhibited different N addition thresholds prior to SMS declines. The critical N addition threshold points were observed at 6.44 and 9.41 g N $m^{-2}$ $year^{-1}$ for topsoil and subsoil, respectively (Figure 2a,b). Similarly, most SMS at different threshold levels, except for the topsoil $SMS_{25\%}$, also showed a hump-shaped response to N addition (Figure 2c,d). In addition, through our constructed ecological service networks, we found that the different individual services are strongly linked within topsoil or subsoil (Figure 2e,f). In the topsoil, the second dimension was primarily driven by PMR, BG activity, TP, AP activity, archaeal richness and pathogen control. In contrast, the first and third dimensions were dominated by microbial functional potential and N cycling processes, respectively, both exhibiting hump-shaped patterns along the N addition gradient (Figure 2e). In addition, we further found that N addition significantly enhanced both the first and second functional dimensions of the subsoil, which are primarily governed by microbial decomposition capacity and mineralization processes, respectively (Figure 2f).

## 3.2 | Effects of N addition on soil microbial co-occurrence networks and ecological clusters

Microbial networks exhibited distinct co-occurrence patterns along the N addition gradient in the topsoil and subsoil (Figure S2). To be specific, topology properties of the microbial network including the number of nodes, number of edges, average path length and complexity in the topsoil and subsoil all displayed a trend that initially increased to a point, followed by a decrease along the N addition gradient, while the average degree and transitivity presented an opposite trend (Figure 3a,b). In addition, network complexity was significantly affected by soil depth, with the topsoil network being far more complex than the subsoil (Figure 3a,b; Table S3). Meanwhile, four key ecological clusters (Clusters #1–4; Table S4) were presented in both topsoil and subsoil microbial co-occurrence networks. All key ecological clusters were dominated by Actinobacteria and Proteobacteria as the main phyla (Figure 3c).

## 3.3 | Linkages of soil multiservices with microbial co-occurrence networks, ecological clusters and environmental factors

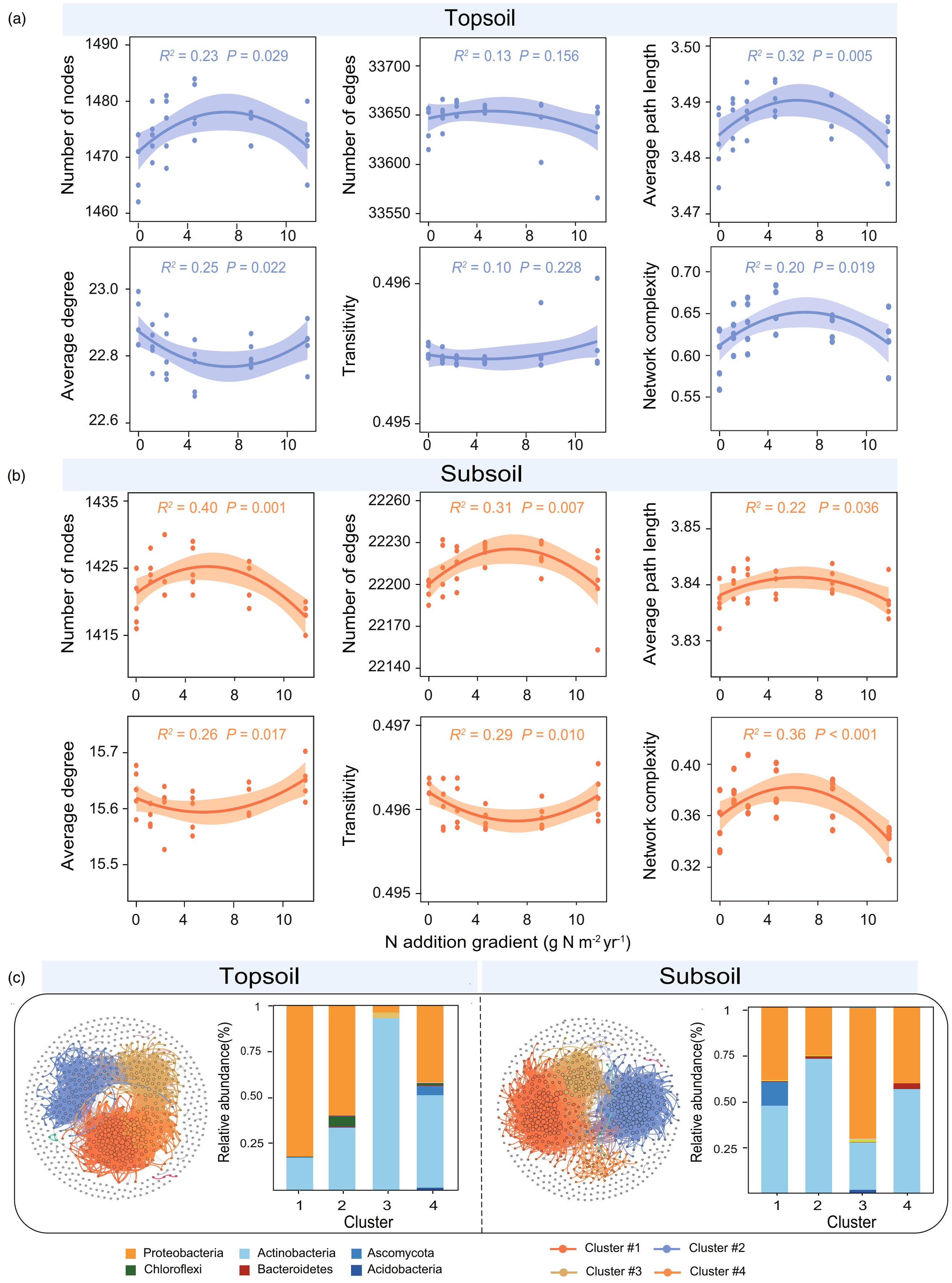
We identified an essential role of the microbial network in predicting dryland soil multiservices in this study (Figures 4 and 5). Specifically, from the topsoil, topology characteristics of microbial networks were significantly correlated with the key individual soil services such as nutrient supply, biodiversity and SOM decomposition, and also with averaged SMS (Figure 4a). In subsoils, microbial network characteristics were significantly associated with nutrient supply, microbial diversity, plant–soil symbionts and $SMS_{\text{Dimension 3}}$ (Figure 4b). Ordinary least squares (OLS) linear regression showed that the network complexity was positively correlated with $SMS_a$ (Figure 5a), $SMS_{50\%}$ (Figure S3) and $SMS_{\text{Dimension 1}}$ (Figure 5c) in the topsoil. In comparison with topsoil, subsoil microbial network complexity was only positively associated with $SMS_a$ and $SMS_{\text{Dimension 3}}$ (Figure 5b,d). Meanwhile, the topology characteristics of microbial networks were closely associated with WHC and Fe/Al oxides in the topsoil and correlated with pH and most mineral properties in the subsoil (Figure 4).

Similar to microbial network properties, and especially complexity, microbial ecological clusters were also crucial for the regulation of soil services and soil multiservices (Figure 6). The average abundance of topsoil microbial ecological clusters (Clusters #3–4) was significantly positively correlated with most soil services (soil C storage, nutrient supply, biodiversity, SOM decomposition and plant–soil symbiosis) and soil multiservices (Figure 6a). In subsoil, only the abundance of Cluster #4 was negatively correlated with some soil services (soil nutrient supply and SOM decomposition), as well as soil multiservices (Figure 6b). More importantly, the abundance of microbial ecological clusters was more closely correlated with plant properties, soil physicochemical properties and mineral protection in the topsoil than that in the subsoil (Figure 6).

## 3.4 | The direct and indirect influence of environment on soil multiservices

The results of the BRT model and SEM analyses collectively demonstrated that the three network dimensions influencing soil multiservices were primarily driven by soil depth (Figures 7 and 8 and Figure S4). The biological factors (microbial ecological clusters abundance and network complexity) were more important in driving dimensions 1 and 2 in the

FIGURE 3 Responses of network topology to N addition gradients. In all panels, filled blue curves and orange curves represent network attributes in the topsoil (a) and subsoil (b), respectively. The curves were fitted using non-linear regression models, with shaded areas representing the 95% confidence intervals. $R^2$ and $p$-values are shown in corresponding colours. (c) Ecological clusters (Clusters #1–4) based on the effects of N addition on soil microbial networks. Different colours represent different network clusters; node sizes are scaled to the relative abundance of the OTUs; the thickness of a connection between nodes represents the magnitude of Spearman's correlation between nodes. The stacked bar charts represent the relative abundance of the microbial phylum (>0.01%) in each ecological cluster in the topsoil and subsoil.


(a)
Topsoil
Number of nodes
R² = 0.23 P = 0.029
1490
1480
1470
1460
Number of edges
R² = 0.13 P = 0.156
33700
33650
33600
33550
Average path length
R² = 0.32 P = 0.005
3.50
3.49
3.48
3.47
Average degree
R² = 0.25 P = 0.022
23.0
22.8
22.6
Transitivity
R² = 0.10 P = 0.228
0.496
0.495
Network complexity
R² = 0.20 P = 0.019
0.70
0.65
0.60
0.55
0
4
8
10
(b)
Subsoil
Number of nodes
R² = 0.40 P = 0.001
1435
1425
1415
Number of edges
R² = 0.31 P = 0.007
22260
22230
22200
22170
22140
Average path length
R² = 0.22 P = 0.036
3.85
3.84
3.83
Average degree
R² = 0.26 P = 0.017
15.7
15.6
15.5
Transitivity
R² = 0.29 P = 0.010
0.497
0.496
0.495
Network complexity
R² = 0.36 P < 0.001
0.40
0.36
0.32
N addition gradient (g N m⁻² yr⁻¹)
(c)
Topsoil
Subsoil
Relative abundance(%)
1
0.75
0.50
0.25
1
2
3
4
Cluster
Proteobacteria
Actinobacteria
Ascomycota
Chloroflexi
Bacteroidetes
Acidobacteria
Cluster #1
Cluster #2
Cluster #3
Cluster #4

topsoil multiservices. With increasing soil depth, the contribution of plant properties to soil multiservices decreased, while the contribution of mineral protection increased (Figure 7). To be specific, for the topsoil, the BRT model revealed that the abundance of microbial cluster #2, pH and $Ca_{exe}$ were most influential for the three dimensions of soil multiservices, respectively, while $Ca_{exe}$, $Mg_{exe}$ and microbial network complexity were the major predictors from subsoil (Figure S4). SEM analysis further showed that dimensions 1 and 3 of the topsoil multiservices were directly modulated most by soil microbial network complexity and ecological cluster abundance, with standardized direct effects of 0.45 and 0.32, respectively (Figure 8a). In contrast, dimensions 2 and 3 of the subsoil multiservices were directly regulated by soil physicochemical properties and microbial network complexity, with standardized direct effects of 0.73 and 0.50, respectively (Figure 8b). Plant properties could have an indirect impact on dimensions 1 and 3 of multiservices by regulating soil mineral protection, microbial ecological clusters abundance and network complexity in the topsoil (Figure 8a), while N addition could regulate soil physicochemical properties and microbial network complexity indirectly regulating dimensions 2 and 3 of multiservices in the subsoil, respectively (Figure 8b). The aforementioned findings were further confirmed by the results of the BRT model and SEM analysis on average and multiple thresholds multiservices (Figure S7).

## 4 | DISCUSSION

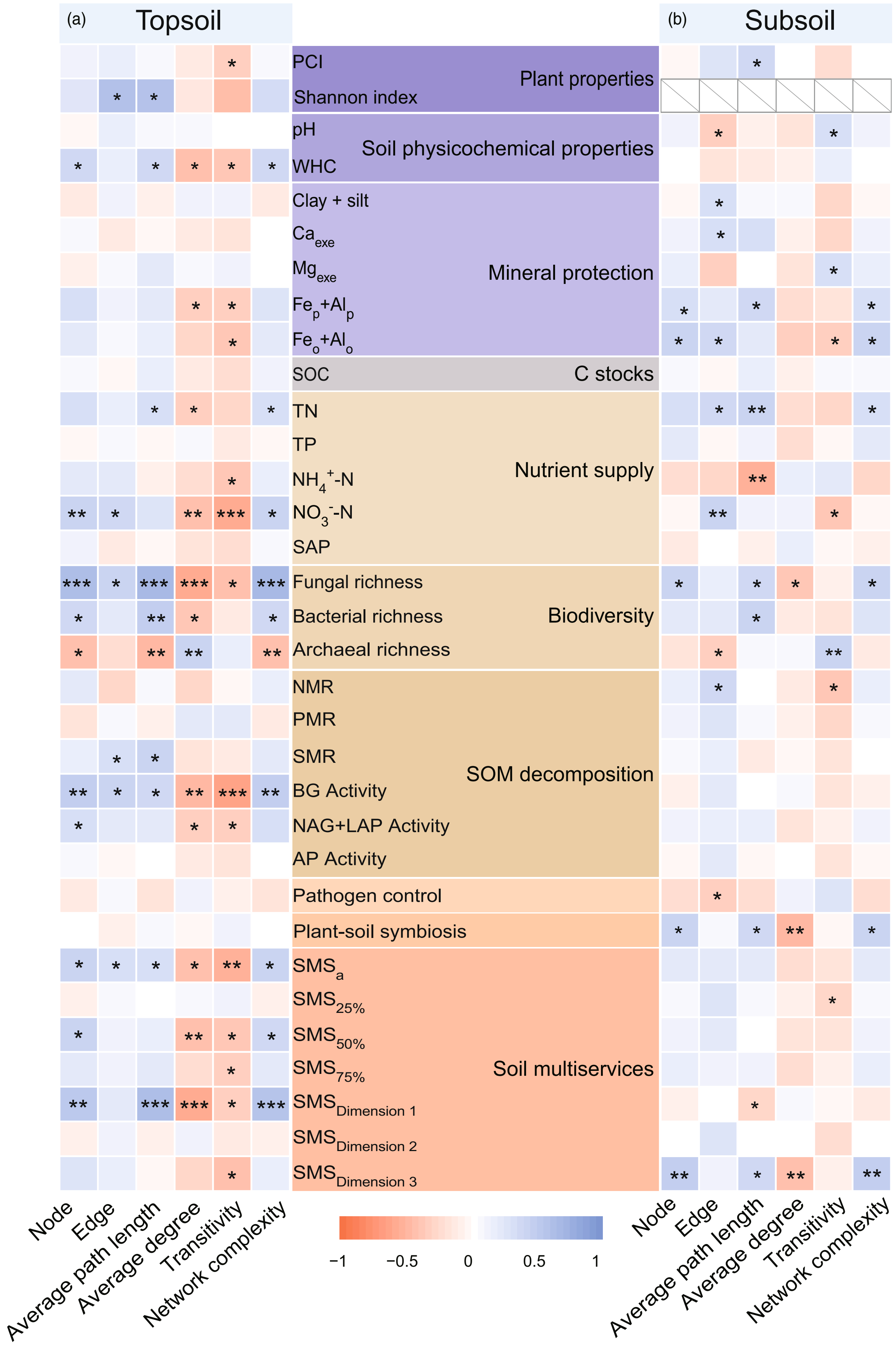
Our study, based on a long-term fertilization experiment conducted in a dryland, provides evidence that multiple ecosystem services follow a hump-shaped pattern in response to N fertilization (Figure 1). This pattern suggests that the intermediate disturbance hypothesis can explain the response of dryland ecosystem services to fertilization or increased atmospheric N deposition, an important and ongoing global change. Moreover, properties of soil microbial networks, particularly network complexity, significantly contribute to explaining the response of multiple ecosystem services to increasing N fertilization. This knowledge is critical for the conservation of drylands in the coming generations.

### 4.1 | Non-linear response of soil multiservices to N increases

Numerous studies have documented significant effects of N addition on soil ecosystem functions and services (Liu et al., 2020; Xing et al., 2022; Yang et al., 2024). Similarly, our results demonstrated that 13 years of N addition significantly affected soil services such as soil C stocks, nutrient supply, SOM decomposition and pathogen control in both topsoil and subsoil (Figure 1A,B,D,E). Specifically, N addition significantly increased soil nutrients required for microbial growth and reproduction, including soil $NH_4^+$-N, $NO_3^-$-N, SAP and SOC at two soil depths (Figure S1). These nutrient changes may affect the decomposition and utilization of soil resources by microbes, driving soil C, N and P cycling (Yuan et al., 2019; Zhang et al., 2025). Consistent with this deduction, soil nutrients were significantly correlated with soil C, N and P mineralization rates and acquisition enzyme activities across N addition treatments (Figure S5), indicating potential synergies among soil multiple services in response to N enrichment. Notably, there was a significant negative relationship between the P mineralization rate and soil nutrients, such as SAP (Figure S5), which might be attributed to changes in the microbial P utilization strategies. These findings suggest that excessive nutrient availability suppress specific microbial functions, revealing inherent trade-offs among soil functions and services beyond commonly observed synergies. Such trade-offs underscore the intrinsic balance within ecosystems, where the enhancement of one function may incur costs to others. In addition, high microbial diversity may promote the proliferation of functionally important microbes, such as arbuscular mycorrhizal fungi and N-fixing bacteria, that can improve plant nutrient acquisition and enhance their disease resistance (Bertola et al., 2021; Odelade & Babalola, 2019). Our finding that greater microbial richness improved the control of fungal pathogens and plant–soil symbiosis metrics in both the topsoil and subsoil further supports this argument (Figure S5), suggesting that biodiversity plays a vital role in maintaining ecosystem stability and resistance, highlighting the synergistic relationships among these services. These findings underscored the importance of considering not only the direct effects of N addition on individual soil functions but also their impact on the inherent linkages among functions, which ultimately shape ecosystem sustainability (Philippot et al., 2021). Taken together, soil multiservices are not merely a collection of independent services but rather an interconnected system driven by trade-offs and synergies among key biogeochemical processes.

Consistent with our first hypothesis, we observed that soil multiservices calculated based on the average, multi threshold and multidimension methods exhibited a hump-shaped response to N addition (Figure 2), indicating that the response of soil multiservices to N addition was controlled by a critical threshold point. Indeed, previous studies have revealed a positive effect of N addition on ecosystem functioning, but such an effect was characterized by a critical

FIGURE 4 The correlation between soil microbial network complexity and soil services in the topsoil (a) and subsoil (b). Panel colours represent Spearman's correlation value. *, ** and *** represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. PCI, plant C input; WHC, water holding capacity (%); $Ca_{exe}$, exchangeable $Ca^{2+}$; $Mg_{exe}$, exchangeable $Mg^{2+}$; $Fe_p + Al_p$, the sum of pyrophosphate-extractable Fe/Al oxides; $Fe_o + Al_o$, the sum of oxalate-extractable Fe/Al oxides. SOC, soil organic C; TP, total phosphorus; TN, total N; $NH_4^+$-N, Ammonium-N; $NO_3^-$-N, nitrate-N; SAP, available inorganic phosphorus; SMR, soil microbial respiration; NMR, the rate of N mineralization; PMR, the rate of P mineralization; BG, β-1,4-glucosidase; NAG, β-1,4-N-acetylamino glucosidase; LAP, leucine aminopeptidase; AP, alkaline phosphatase.


(a) Topsoil
(b) Subsoil
PCI
Shannon index
Plant properties
pH
WHC
Soil physicochemical properties
Clay + silt
$Ca_{exe}$
$Mg_{exe}$
$Fe_p+Al_p$
$Fe_o+Al_o$
Mineral protection
SOC
C stocks
TN
TP
$NH_4^+$-N
$NO_3^-$-N
SAP
Nutrient supply
Fungal richness
Bacterial richness
Archaeal richness
Biodiversity
NMR
PMR
SMR
BG Activity
NAG+LAP Activity
AP Activity
SOM decomposition
Pathogen control
Plant-soil symbiosis
$SMS_a$
$SMS_{25\%}$
$SMS_{50\%}$
$SMS_{75\%}$
$SMS_{Dimension\ 1}$
$SMS_{Dimension\ 2}$
$SMS_{Dimension\ 3}$
Soil multiservices
Node
Edge
Average path length
Average degree
Transitivity
Network complexity
−1
−0.5
0
0.5
1

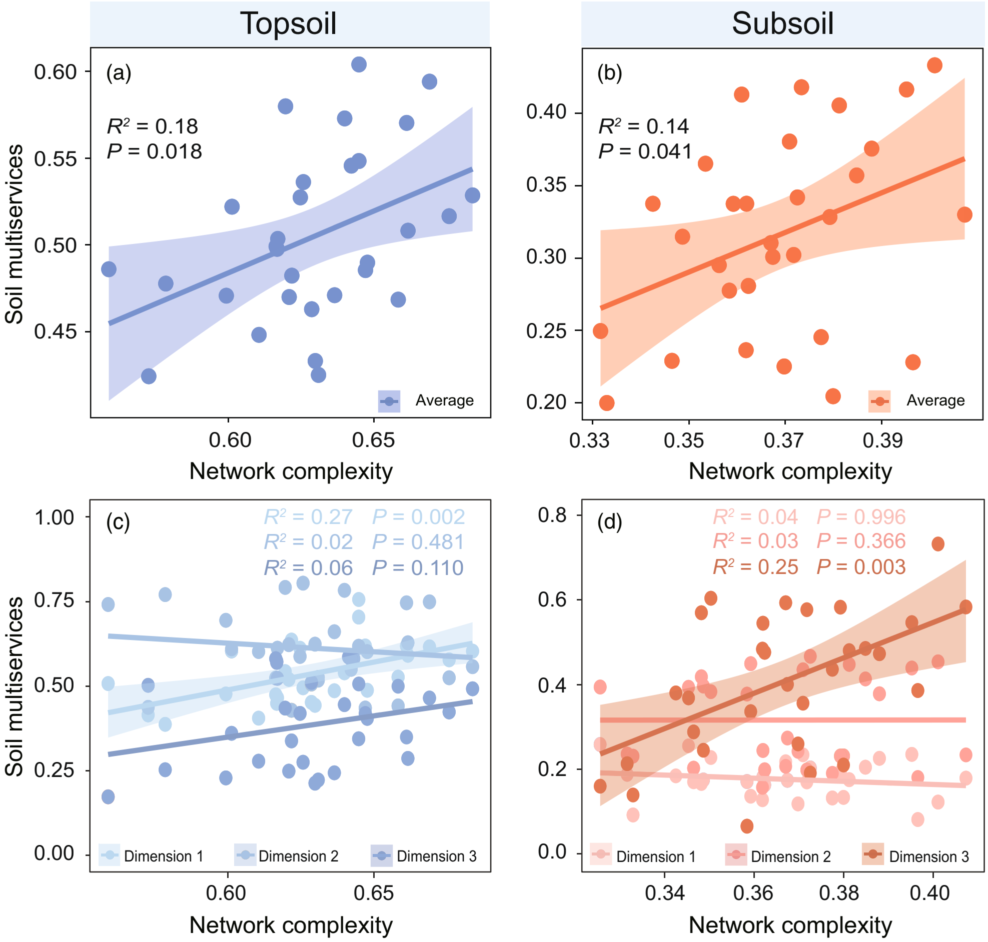


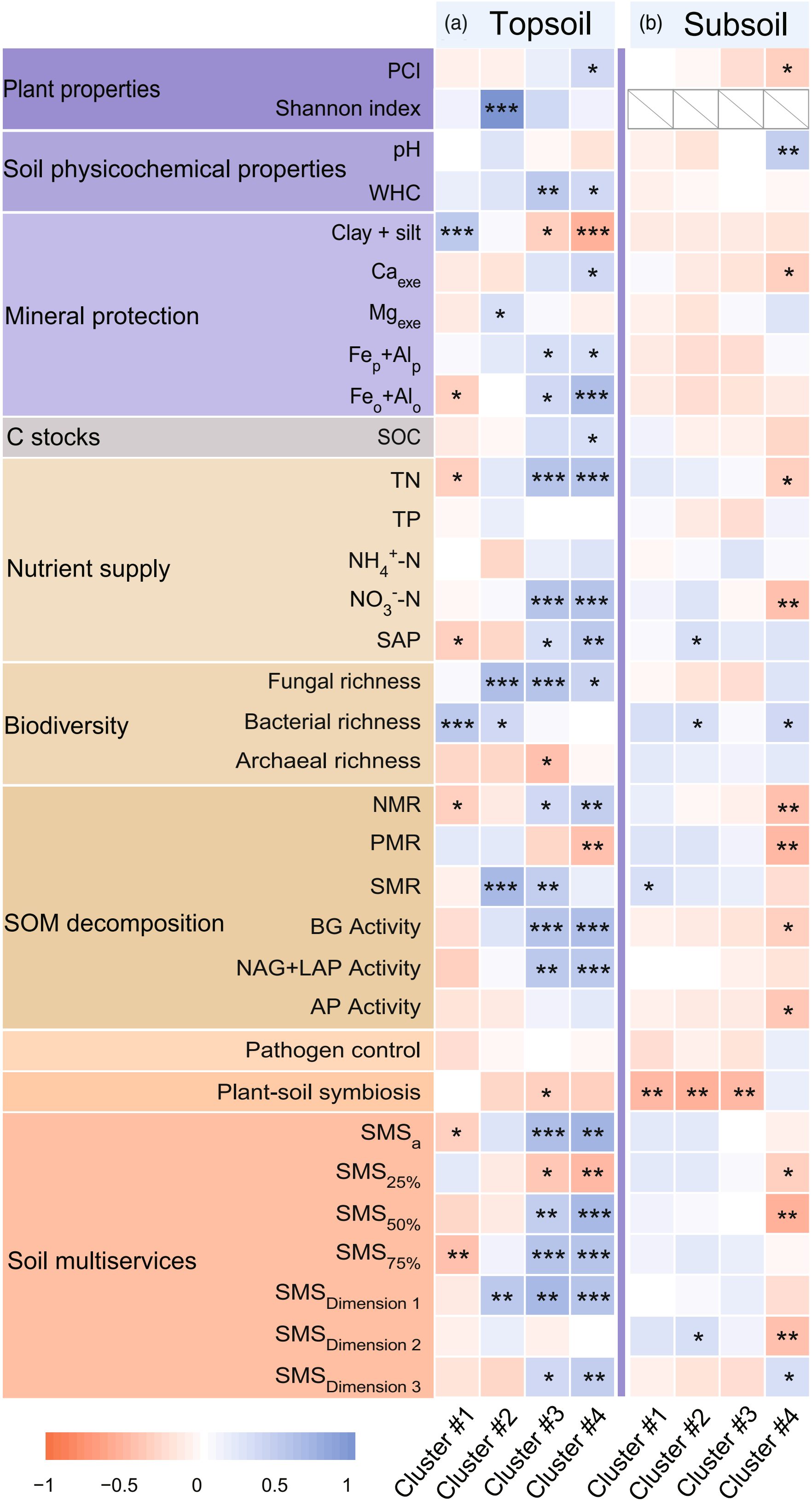
**FIGURE 5** The linear relationships between soil averaged (a, b) or dimensional multiservices (c, d) and network complexity. Solid lines were fitted using least squares regression, while the shaded areas indicate 95% confidence intervals. $R^2$ and *p*-values are shown for the averaged or dimensional multiservices.

threshold (de Vries et al., 2014; Wu et al., 2023). This response is in agreement with the intermediate disturbance hypothesis. Under the N-limited conditions typical of many temperate ecosystems, low levels of N input tend to stimulate soil microbial growth and activity (Feng et al., 2022; Spohn et al., 2016) and enhance services such as soil biodiversity or nutrient supply (Feng et al., 2022; Yuan et al., 2019), thereby improving soil multiservices. But after passing a critical threshold (de Vries et al., 2014; Wu et al., 2023), excessive N addition can result in drastic declines of soil multiservices through various mechanisms including soil acidification, release of mineral-associated aluminium and loss of base cations, which together can then inhibit microbial growth, activity, nutrient supply processes and plant–soil interactions (Lucas et al., 2011; Wu et al., 2023; Ye et al., 2017). These arguments are further supported by the results of our study, which reveal significant reductions in soil pH (Figure S8d), extracellular enzyme activities (Figure S1m–o), microbial respiration (Figure S1l), and soil N and P mineralization processes (Figure S1j–k) under high N additions. Notably, compared with the other soil multiservice dimensions, topsoil dimension 2 decreased with increasing levels of N addition (Figure 2e). This may be attributed to the fact that dimension 2 was closely associated with the cycling and transport of P among plant, soil and microbes (Figure S9c). Our finding is further supported by a recent study conducted in a Mediterranean forest ecosystem disturbed by logging (Zhou et al., 2023). These results suggest that the different components of soil multiservices can exhibit divergent responses to environmental disturbance, but ultimately result in a balance among these services forming a distinguishable response pattern.

### 4.2 | Soil microbial network complexity and ecological clusters regulating soil multiservices responses

Consistent with our second hypothesis, there was a significant relationship between soil multiservices and the complexity of the microbial network created by fungi, bacteria and archaea (Figures 4 and 5). Generally, a more complex microbial co-occurrence network indicates a greater variety of structural traits among microbial groups, which enhances the division of metabolic labour and increases species turnover patterns (De Gannes et al., 2015; Odelade & Babalola, 2019). This complexity may promote niche overlap,

FIGURE 6 The correlation between ecological clusters (Clusters #1–4) abundances formed by highly correlated OTUs in soil microbial networks and soil services in the topsoil (a) and subsoil (b). Panel colours represent Spearman's correlation value. *, ** and *** represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.



resource competition and inter-species interactions, boosting resource use efficiency (Liu et al., 2025; Naeem et al., 1994) and ultimately enhancing soil multiservices, such as nutrient supply, plant pathogen control and plant–soil symbiosis (Delgado-Baquerizo et al., 2020; Jiao, Lu, et al., 2022; Zhang et al., 2025). Additionally, a complex microbial network reflects greater self-regulation capacity, including higher resistance to environmental changes and greater resilience to disturbances (Yuan et al., 2021). These properties can

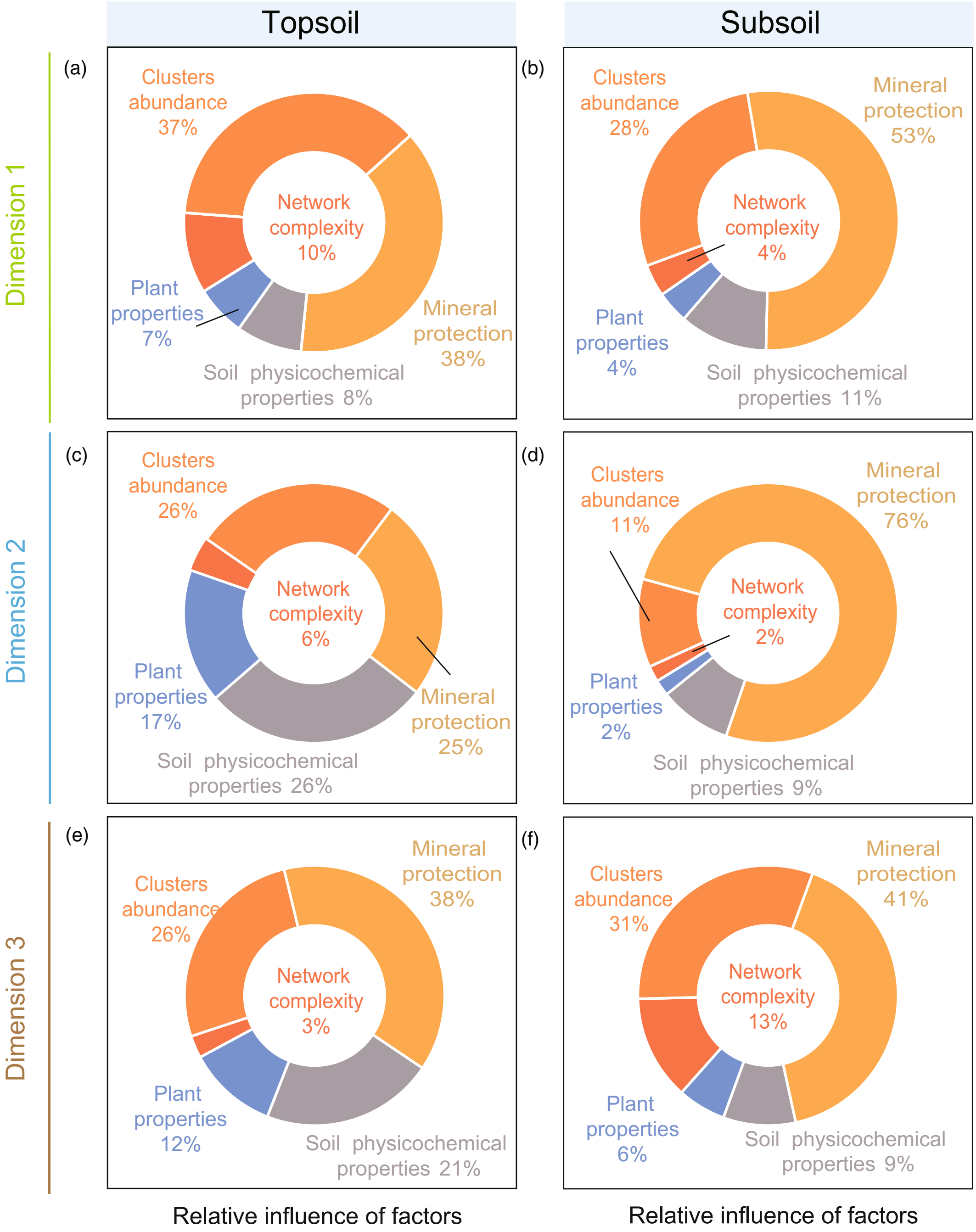


FIGURE 7 The relative contribution (%) of plant properties, soil physicochemical properties, mineral protection, microbial clusters abundance and microbial network complexity for the BRT model of soil multiservices dimensions in the topsoil (a, c, e) and subsoil (b, d, f). Plant properties include: Shannon index and plant C input; soil physicochemical properties include: pH and water holding capacity (%); mineral protection includes: clay+silt, exchangeable $Ca^{2+}$, exchangeable $Mg^{2+}$, the sum of pyrophosphate-extractable Fe/Al oxides and the sum of oxalate-extractable Fe/Al oxides; clusters abundance includes: microbial ecological clusters #1–4. See Figure S4 for details.

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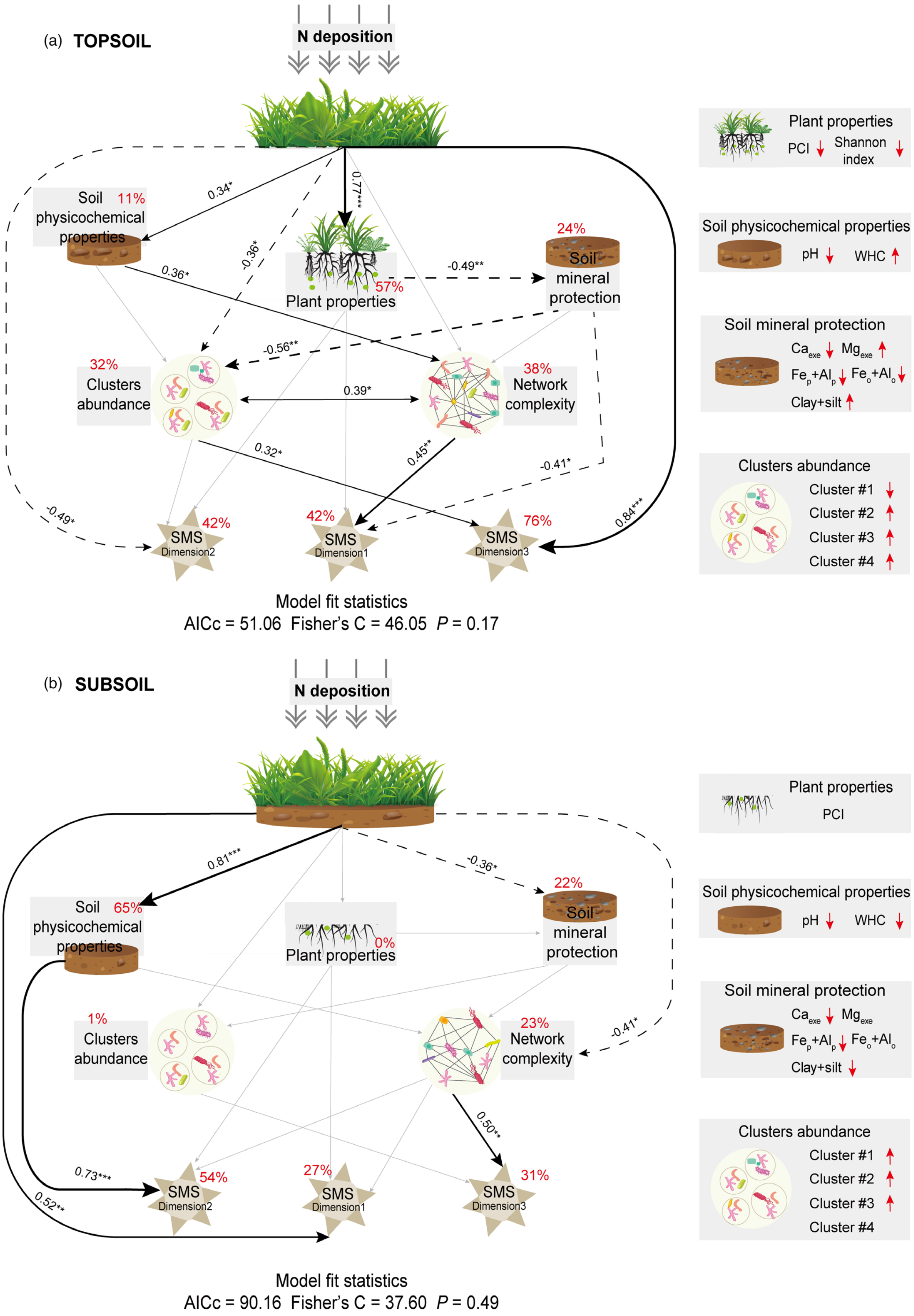
FIGURE 8 Structural equation modelling (SEM) analysis of direct and indirect effects of N addition on soil multiservices (SMS) of different dimensions in the topsoil (a) and subsoil (b). Black solid and dashed arrows indicate positive and negative relationships, respectively, with thickness proportional to standardized path coefficients, indicating path strengths. Grey arrows indicate insignificant relationships. The multiple-layer rectangles indicate the PC1 from the principal components analysis (PCA) of soil physicochemical properties, mineral protection and microbial clusters abundance. Percentages in red adjoining the rectangles indicate the variance explained for the variable in the model ($R^2$). The symbols '↑' and '↓' beside the variables represent a significant increase or decrease relationship between the variables and the first component from the PCA, respectively. *, ** and *** represent significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.


(a) TOPSOIL
N deposition
Soil physicochemical properties 11%
Plant properties 57%
Soil mineral protection 24%
Clusters abundance 32%
Network complexity 38%
SMS Dimension2 42%
SMS Dimension1 42%
SMS Dimension3 76%
0.34*
0.77***
-0.36*
0.36*
-0.49**
-0.56**
0.39*
0.32*
0.45**
-0.41*
-0.49*
0.84***
Model fit statistics
AICc = 51.06 Fisher's C = 46.05 P = 0.17
Plant properties
PCI ↓ Shannon index ↓
Soil physicochemical properties
pH ↓ WHC ↑
Soil mineral protection
Ca$_{exe}$ ↓ Mg$_{exe}$ ↑
Fe$_p$+Al$_p$ ↓ Fe$_o$+Al$_o$ ↓
Clay+silt ↑
Clusters abundance
Cluster #1 ↓
Cluster #2 ↑
Cluster #3 ↑
Cluster #4 ↑
(b) SUBSOIL
N deposition
Soil physicochemical properties 65%
Plant properties 0%
Soil mineral protection 22%
Clusters abundance 1%
Network complexity 23%
SMS Dimension2 54%
SMS Dimension1 27%
SMS Dimension3 31%
0.81***
-0.36*
-0.41*
0.50**
0.73***
0.52**
Model fit statistics
AICc = 90.16 Fisher's C = 37.60 P = 0.49
Plant properties
PCI
Soil physicochemical properties
pH ↓ WHC ↓
Soil mineral protection
Ca$_{exe}$ ↓ Mg$_{exe}$
Fe$_p$+Al$_p$ ↓ Fe$_o$+Al$_o$
Clay+silt ↓
Clusters abundance
Cluster #1 ↑
Cluster #2 ↑
Cluster #3 ↑
Cluster #4

mitigate changes in soil services and help maintain higher levels of soil multiservices (Liu et al., 2025; Wagg et al., 2019). In addition, our results further revealed that most of the ecological clusters with Actinobacteria and Proteobacteria as the dominant bacteria in the topsoil and subsoil under N addition were significantly correlated with key soil services such as nutrient supply, SOM decomposition, biodiversity and multiservices (Figure 6). This may be explained by the fact that many Proteobacteria and Actinobacteria are characterized as copiotrophs, with potential for greater nutrient utilization effectiveness and metabolic modulation of ecological processes (Dai et al., 2018; Fierer et al., 2007), which may be driving observed increases in soil multiservices (Jiao, Lu, et al., 2022; Jiao, Qi, et al., 2022).

We found that the response of topsoil multiservices was significantly greater than that of subsoil multiservices (Figure 2, Table S2), which is consistent with previous findings (Wang, Li, et al., 2023). It is crucial to recognize that both plant properties and soil mineral properties play indispensable roles in mediating soil multiservices in the face of N enrichment along the soil profile. Compared to the subsoil, we inferred that N addition could increase inputs of accessible plant-derived compounds (leaf and root litter as well as root secretions) into the topsoil, providing more C and nutrient availability to microbes (Morrison et al., 2018; Treseder, 2008). Such modifications could promote the transition of microbial communities from more oligotrophic to more copiotrophic taxa (Proteobacteria and Actinobacteria). This shift in microbial community structure was directly related to the regulation of SOM decomposition processes and nutrient supply, which, in turn, enhanced microbial catabolism/anabolism, as well as pathogen control through the activation of beneficial microbes (Bertola et al., 2021; Odelade & Babalola, 2019). Consistent with this deduction, the results of SEM analysis revealed that N addition affected microbial network complexity and ecological cluster abundance via modifications to plant properties, indirectly regulating dimensions 1 and 3 in the topsoil multiservices (Figure 8a), which were mainly associated with soil C stocks, SOM decomposition and nutrient supply services. These findings further supported our second hypothesis regarding N addition effects on multiservices to be indirectly regulated by soil biodiversity and microbial network complexity, especially in dryland topsoils.

In addition, the increase in the content of exchangeable cations (such as $Ca_{exe}$, $Mg_{exe}$) and clay minerals along the soil profile (Figure S8f–j) can prevent microbial processing of SOM by forming organo-mineral associations via physicochemical adsorption (Chen et al., 2021; Lalonde et al., 2012). Previous studies have found that N addition-induced soil acidification could increase cation ($Ca_{exe}$, $Mg_{exe}$) leaching as well as iron ($Fe^{3+}$) and aluminium ($Al^{3+}$) solubility (Lucas et al., 2011), which could further cause SOC desorbing from mineral surfaces, leading to subsequent degradation and thereby enhancing soil nutrient supply (Feng et al., 2022). As expected, BRT analysis suggested that the relative contribution of mineral protection to different dimensions of soil multiservices enhanced with soil depth (Figure 7 and Figure S4). However, in contrast with the result of BRT analysis, SEM analysis demonstrated that N addition directly improved dimension 3 of multiservices via influencing microbial network complexity rather than mineral protection in the subsoil (Figure 8b). This may be due to the fact that nutrients and other resources in the subsoil are scarce, resulting in lower microbial activity; simultaneously, most of SOM is adsorbed on active mineral surfaces, making microbial utilization even more difficult at greater depths (Schmidt et al., 2011). Under such conditions, instead of willingly expending large amounts of energy to break down organo-mineral associations to acquire nutrients, microbes may prefer to consume more available soil nutrients to maintain growth and reproduction, ultimately altering biodiversity and plant–soil symbiosis services (Schimel & Weintraub, 2003; Schmidt et al., 2011; Sokol et al., 2022). These results suggest that both plant traits and mineral-associated protection are crucial to regulating the contrasting responses of topsoil and subsoil multiservices to long-term N addition.

Importantly, other than plant properties and mineral protection, soil physicochemical properties like soil pH and water holding capacity can also play crucial roles in regulating topsoil and subsoil multiservices in response to N addition, including soil C stocks, SOM decomposition, nutrient supply and pathogen control services (Figure S10). In drylands, greater soil water holding capacity promotes microbial activity by improving plant litter decomposition, enhancing substrate diffusion and oxygen content, thus increasing SOC decomposition, nutrient supply and ultimately improving soil multiservices (Scherzinger et al., 2024). Overall, our findings highlight that in topsoil, N addition-induced shifts in plant properties and soil physicochemical properties can enhance soil multiservices, but mostly via changes in microbial network complexity and ecological clustering. In contrast, subsoil multiservices were more strongly regulated by soil physicochemical properties and microbial networks.

## 5 | CONCLUSIONS

Based on a long-term experiment, we found that the responses of dryland soil ecosystem services to fertilization strongly align with the intermediate disturbance hypothesis. Small increases in N can promote ecosystem services in nutrient poor drylands, but excess N may collapse the capacity to maintain important ecosystem services. This concept may be applied to any limiting resource, and the strong regulatory role of soil microbial networks observed in our study deserves further attention across environmental change factors and ecosystems. We provide new evidence that soil microbial network properties such as overall network complexity modulate the effects of N fertilization on ecosystem services, especially for topsoils. This knowledge is critical to maintaining the sustainability of terrestrial ecosystems and suggests that the promotion of microbial diversity and preservation of topsoils will be incredibly important to mitigate effects of N deposition, especially under more diverse and more frequent global climate change impacts in the future.

While microbial network analysis offers a powerful mathematical framework, it captures statistical correlations rather than direct biological interactions. Further manipulative experiments are needed to uncover the specific mechanistic roles of individual microbial taxa. Moreover, soil biota are extremely complex and diverse, ranging from fungi, bacteria, archaea, protists and invertebrates, composing complex soil food webs that can jointly drive multiple ecosystem functions and services (Manlick et al., 2024; Shi et al., 2024). Therefore, future studies are encouraged to explore how N deposition-induced changes within and among these multitrophic levels can regulate soil multiservices, which could enhance our understanding of the relationships between the totality of soil biota and related soil ecosystem services in the future.

## AUTHOR CONTRIBUTIONS

Xiaobo Yuan, Manuel Delgado-Baquerizo, Guiyao Zhou, Hua Fu, Decao Niu and Ying Wang conceived the ideas; Yaodan Zhang, Hua Fu and Xiaobo Yuan designed the methodology; Yaodan Zhang, Ying Wang, Guiyao Zhou, Daniel Revillini, Huiying Liu, Ning Chen, Baoming Du, Ding Guo, Decao Niu and Shujuan Wu analysed the data; Yaodan Zhang, Ying Wang, Jingrun Xu and Qingwei Li performed the lab analysis; Yaodan Zhang, Guiyao Zhou, Manuel Delgado-Baquerizo, Hua Fu, Xiaobo Yuan and Ying Wang wrote the original draft; Yaodan Zhang, Daniel Revillini, Huiying Liu, Manuel Delgado-Baquerizo, Decao Niu, Hua Fu and Xiaobo Yuan wrote review and editing; All authors contributed critically to the drafts and gave final approval for publication.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70112.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in Dryad Digital Repository at http://doi.org/10.6084/m9.figshare.28580120 (Zhang, 2025).

## ORCID

*Yaodan Zhang* https://orcid.org/0009-0001-5193-4917
*Guiyao Zhou* https://orcid.org/0000-0002-1385-3913
*Daniel Revillini* https://orcid.org/0000-0002-0380-4106
*Huiying Liu* https://orcid.org/0000-0001-8903-6103
*Ning Chen* https://orcid.org/0000-0002-1779-915X
*Baoming Du* https://orcid.org/0000-0002-1020-0642
*Decao Niu* https://orcid.org/0000-0003-2607-043X
*Hua Fu* https://orcid.org/0000-0003-1526-9304
*Xiaobo Yuan* https://orcid.org/0009-0001-4834-238X

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** The 17 individual soil variables used for calculating soil multiservices. Categories, units and a brief description are given for each variable.

**Table S2.** Results of two-way ANOVA analysis for soil services and multiservices as dependent on N addition, soil depth and their interactions. The *F*-ratios are shown together with their level of significance.

**Table S3.** Results of two-way ANOVA analysis for soil microbial network complexity as dependent on N addition, soil depth and their interactions. The *F*-ratios are shown together with their level of significance.

**Table S4.** Topological properties of soil microbial ecological clusters in the topsoil and subsoil.

**Figure S1.** Effects of N addition (N), soil depth (D) and their interactions (N × D) on individual soil variables.

**Figure S2.** Visualization of soil microbial co-occurrence networks from $N_0$ to $N_{13.80}$ in the topsoil (a) and subsoil (b).

**Figure S3.** The relationships between network complexity and the multi-threshold services in the topsoil and subsoil.

**Figure S4.** The relative contribution (%) of predictor factors for the BRT model of soil multiservices of different dimension in the topsoil (a, c, e) and subsoil (b, d, f).

**Figure S5.** Correlation coefficients between individual soil variables in the topsoil (a) and subsoil (b).

**Figure S6.** Initial conceptual model of the effects of N addition on multiple soil services and their underlying regulatory mechanisms.

**Figure S7.** Driving factors of soil multiservices of averaged ($SMS_a$) and different thresholds ($SMS_{25\%}$, $SMS_{50\%}$ and $SMS_{75\%}$) in the topsoil and subsoil.

**Figure S8.** Effects of N addition, soil depth and their interactions on plant properties (a–c), soil physicochemical properties (d, e) and mineral protection (f–j).

**Figure S9.** The linear relationships between soil variables and multiservices in different dimensions.

**Figure S10.** Associations of soil multiservices with abiotic factors (plant properties, soil physicochemical properties and mineral protection) in the topsoil (a) and subsoil (b).

**Figure S11.** Effects of N addition on soil multiservices in the topsoil (a) and subsoil (b).

**Figure S12.** The within-module (Zi) and among-module (Pi) connectivity scores of the nodes in the co-occurrence networks of the topsoil (a) and subsoil (b).

**Figure S13.** Effects of N addition on Shannon diversity of fungi (a, d), bacteria (b, e) and archaea (c, f) in the topsoil and subsoil.

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