Effects of defoliation and nitrogen on carbon dioxide (CO_2) emissions and microbial communities in soils of cherry tree orchards

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ABSTRACT

Background. In farmland, microbes in soils are affected by exogenous carbon, nitrogen, and soil depth and are responsible for soil organic carbon (SOC) mineralization. The cherry industry has been evolving rapidly in northwest China and emerged as a new source of income for local farmers to overcome poverty. Accordingly, it is highly imperative to probe the effect of defoliation and nitrogen addition on carbon dioxide (CO_2) emissions and microbial communities in soils of dryland cherry orchards.

Methods. CO_2 emissions and microbial communities were determined in soil samples at three depths, including 0–10 cm, 10–30 cm, and 30–60 cm, from a 15-year-old rain-fed cherry orchard. The samples were respectively incubated with or without 1% defoliation under three input levels of nitrogen (0 mg kg^{-1}, 90 mg kg^{-1}, and 135 mg kg^{-1}) at 25°C in the dark for 80 days.

Results. Defoliation and nitrogen addition affected CO_2 emissions and microbial communities and increased microbial biomass carbon (MBC), the activity of soil catalase, alkaline phosphatase, and cellulase in soils of the dryland cherry orchard. The culture with defoliation significantly promoted CO_2 emissions in soils at the three depths mainly by increasing the MBC, catalase, alkaline phosphatase, and cellulase activities, resulted in positive priming index. Nitrogen addition elevated the MBC and changed soil enzymes and reduced CO_2 emissions in soils at the three depths. Moreover, the priming index was higher in deep soils than in top and middle soils under the condition of defoliation and nitrogen addition. No significant differences were observed in the soil bacterial diversity (Chao1, Shannon, and Simpson) among all treatments. Meanwhile, the relative abundance of *Proteobacteria* was markedly increased and that of *Acidobacteria* was substantially diminished in soils at the three depths by defoliation and nitrogen addition. The results sustained that defoliation and nitrogen can regulate SOC dynamics by directly and indirectly affecting soil microbial activities and communities. As a result, the combination of defoliation return and nitrogen fertilization management is a promising strategy to increase SOC and promote soil quality in dryland cherry orchards.

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INTRODUCTION

Soil respiration is the primary mode of carbon dioxide (CO_2) exchange between the soil carbon pool and atmosphere and one of the largest fluxes in the carbon cycle of terrestrial ecosystems (78–98 Gt C per year) (*Bond-Lamberty et al., 2018*; *Tang et al., 2020*). Accordingly, soil respiration can alter CO_2 in the atmosphere and carbon storage in soils, thus affecting the global carbon cycle (*Wang et al., 2019*). As a vital component of land respiration in agricultural and forestry ecosystems, soil respiration is readily influenced by vegetation types, cultivation, fertilization, and other active jamming factors (*Buchmann, 2000*; *Raich & Tufekcioglu, 2000*; *Mo et al., 2021*). Reportedly, exogenous carbon inputs affect soil nutrient availability, increase microbial activities and quantity, and change the activity of soil enzymes, thus contributing to the priming effect of CO_2 emissions (*Blagodatskaya & Kuzyakov, 2008*; *Chen et al., 2015*; *Li et al., 2018*; *Liang et al., 2022*). Most of the existing studies focused on the association of soil carbon dynamics and microbial communities with labile carbon inputs (*Blagodatskaya et al., 2007*; *Tian et al., 2016a*; *Li et al., 2017*; *Shahbaz et al., 2018*; *Zhou et al., 2021*). In addition, the effects of complex polymerized organism inputs (such as straw and litter) on soil carbon dynamics and microbial communities have been analyzed mainly in farmland and forestry ecosystems (*Moorhead, Sinsabaugh & Moorhead, 2006*; *Aye et al., 2018*; *Fang et al., 2018b*; *Mo et al., 2021*) but rarely in orchard ecosystems (*Zhou et al., 2021*). Therefore, the study of defoliation inputs to orchard soils can objectively and truly reflect the characteristics of soil CO_2 emissions and microbes in the orchard industry.

Nitrogen fertilizer plays a pivotal role in the management of orchards, which can increase soil nutrients and change microbial activities and communities (*Neff et al., 2002*; *Malhi et al., 2003*; *Leff et al., 2015*; *Wang, Liu & Bai, 2018*), and affect soil respiration (*Razanamalala et al., 2018*; *Sawada, Inagaki & Toyota, 2021*; *Na et al., 2022*). Of note, some hypotheses have been proposed on the mechanism of nitrogen. For instance, the theory of "Microbial stoichiometric decomposition" (*Hessen et al., 2004*; *Chen et al., 2014*; *Cui et al., 2020*) demonstrates that exogenous substance inputs can contribute to high microbial activities and organic matter decomposition, which simulate high CO_2 emissions, by meeting the carbon/nitrogen demand of soil microbes, indicating that high nitrogen availability (abundant nutrients) may favor soil organic matter (SOM) decomposition. The "microbial nitrogen mining" theory shows that soil microbes decompose SOM with labile carbon as an energy source to obtain the required nitrogen and then induce carbon priming effects under low nitrogen conditions (*Moorhead, Sinsabaugh & Moorhead, 2006*; *Borrajo et al., 2011*; *Mason-Jones, Schmücker & Kuzyakov, 2018*; *Na et al., 2022*; *Craine, Morrow & Fierer, 2007*), illustrating that low nitrogen availability (poor nutrients) stimulates SOM decomposition. Despite the obvious odds between the two theories, their mechanisms are closely related to organic carbon, which suggests an inherently critical association of soil carbon respiration with carbon and nitrogen inputs. In this context, there is a need to explore the impacts of exogenous defoliation inputs on CO_2 emissions and microbial mechanisms under different nitrogen levels, thus providing more data and theoretical support for understanding the soil carbon cycle.

Responses of soil CO_2 emissions to different environments are variable because of different soil environments and physicochemical characteristics in soils at varying depths (*De Graaff et al., 2014*; *Tian et al., 2016b*; *Liao et al., 2020*). In top soils (≤10 cm), large amounts of SOC are produced and CO_2 emissions are increased because of litter, fertilization, soil fungi, bacteria, and animals (*Blanco-Canqui & Lal, 2008*; *Tian et al., 2016b*; *Banfield et al., 2018*). Conversely, deep soils can sequestrate more exogenous carbon than top soils because of low SOC (*Fontaine et al., 2007*; *Wang et al., 2014*). Consequently, it is urgent to investigate whether soil respiration in soils at different depths is affected by defoliation and nitrogen additions, thereby providing data to support the development of soil carbon sequestration science.

The cherry industry is highly economically profitable, which has contributed to its rapid growth in northwest China. In this context, this industry has become a new source of income for local farmers to shake off poverty. Accordingly, it is necessary to explore microbial mechanisms of CO_2 emissions in dryland cherry orchards with defoliation and nitrogen addition, thus providing more data and theoretical support for sustainable development of cherry industry.

In this study, soils at different depths were collected from a rain-fed cherry orchard in northwest China for an indoor incubation experiment was performed to ascertain the microbial mechanism regulating CO_2 emissions under defoliation and nitrogen addition. Then, the following three hypotheses were proposed: (1) defoliation and nitrogen addition promoted CO_2 emissions; (2) CO_2 emissions were strongly associated with microbial activities and community; (3) microbial communities varied in soils at different depths.

MATERIAL AND METHODS

Soil collection

Soil samples were obtained at three depths (0–10 cm [top soils], 10–30 cm [middle soils], and 30–60 cm [deep soils]) of the botanical garden test site at Tianshui Normal University (Tianshui, Gansu, China; 34°34′10″N and 105°41′47″E) in 2021. The test site was built in 2002, where the cherry rootstock was Gisela 5 and the cherry variety was Provence. The basic physical and chemical properties of top, middle, and deep soils were as follows: pH: 6.7, 7.8, and 8.1; total organic carbon: 14.74 g kg^{-1}, 12.54 g kg^{-1}, and 7.92 g kg^{-1}; total nitrogen: 0.74 g kg^{-1}, 0.69 g kg^{-1}, and 0.46 g kg^{-1}; available phosphorus: 4.93 mg kg^{-1}, 5.08 mg kg^{-1}, and 3.07 mg kg^{-1}; available potassium: 153.0 mg kg^{-1}, 148.0 mg kg^{-1}, and 100.6 mg kg^{-1}.

Experimental design

The incubation experiment was conducted as a complete factorial experiment of soils at three depths (top, middle, and deep soils) * two kinds of defoliation addition (no-defoliation [C_0] and defoliation [C_A, 1%]) * addition of three levels of nitrogen (0 mg kg^{-1} [N_0], 90 mg kg^{-1} [N_L], and 135 mg kg^{-1} [N_H]) in three replicates with a fully randomized design. Total organic carbon was 451 g kg^{-1} and total nitrogen was 12.47 g kg^{-1} in defoliation.

Incubation tanks (1 L) were respectively added with 100 g soils (dry weight) at different depths for 7 days of pre-incubation at 25 °C (*Mo et al., 2021*). After that, soils were

fully mixed with defoliation according to different treatments. Nitrogen and phosphate fertilizers were dissolved in distilled water and added to soils as a solution. Next, the soils were incubated at 25 °C with the moisture of 60% and the bulk density of 1.2 g cm^{-3} in the dark for 80 days.

Gas collection and soil sampling

For each treatment, three tanks were taken out at 1, 4, 13, and 80 days. The soils in the incubation tanks were stored at −20 °C for detecting microbial biomass carbon (MBC), soil enzyme activities, and microbial communities. Specifically, MBC was measured with the chloroform-fumigation extraction method (*Vance, Brookes & Jenkinson, 1987*). The activity of alkaline phosphatase was examined with the method of Tabatabai and Bremner (*Tabatabai & Bremner, 1969*) as described in a prior study (*Miralles et al., 2012*). The activity of cellulase was detected using the method of Xu and Zheng (*Xu & Zheng, 1986*). The activity of catalase was determined by titrating 0.1 mol L^{-1} $KMnO_4$ (*Guan, Zhang & Zhang, 1986*).

DNA extraction and high-throughput sequencing

Total DNA was extracted from soils with the Fast DNA SPIN Kit for Soil and FastPrep-24 nucleic acid Extraction instrument (MP Biomedicals, Santa Ana, CA, USA) on the 4th day. The DNA was examined with 1% agarose gel electrophoresis, and its concentration was measured with NanoDrop 2000. The DNA was stored in a refrigerator at −20 °C for subsequent use. The stock solution of the DNA was diluted to about 5 mg/L as the template of polymerase chain reaction (PCR) amplification. Afterward, the 16S rRNA variable regions V3-V4 of bacteria were subjected to PCR amplification with the following primers: CCTAYGGGRBGCASCAG (341F) and GGACTACNNGGGTATCTAAT (806R). The PCR was conducted with a system of 50 μL, including 5 μL of 10 * buffer, 4 μL of dNTP, 0.5 μL of RTAQ (Takara), 1 μL of 10 μmol L^{-1} each front and rear primers, 36.5 μL of ddH_2O, and 2 μL of template DNA, and the detection of each sample was repeated three times. The amplification conditions were as follows: pre-denaturation at 98 °C for 30 s, 30 cycles of chain disassembly at 98 °C for 10 s, annealing at 55 °C for 15 s, and extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. The products obtained by three repeats of DNA amplification were mixed and tested with 1% agarose gel electrophoresis. The concentration of the obtained bacterial PCR products was measured with the PicoGreen kit. After the products were evenly mixed, DNA was purified and recovered with the DNA purification kit (TIANGEN Biotech, Beijing, China). Bacterial 16S rDNA was sequenced by Shenzhen Weicomeng Technology Group Co., Ltd. (Shenzhen, China) with the Novaseq 6000 PE 250 platform.

According to corresponding barcodes, the samples were subjected to paired reading, and then their barcode and primer sequence were removed. The reads at both ends were combined with the FLASH (V1.2.7) software. The QIIME data analysis package was utilized to remove low-quality raw sequences (length < 250 bp, ambiguous base "N", and the average base quality score less than 20). The chimeric sequence was discarded with the MAARJAM database (https://maarjam.ut.ee/). The valid readings were assigned

to operational taxon units or virtual taxa with an identity threshold of 97%, and the representative sequences were identified with SILVA (V128, http://www.arb-silva.de) and MAARJAM databases.

Calculation

CO_2 emissions (mg CO_2 kg_{soil}^{-1}) were calculated as per the titration results of hydrochloric acids.

$$CO_2 = \frac{(V_0 - V) \times C_{HCl}}{2} \times 12 \times \frac{1}{m(1-a)} \times 1000.$$

In this formula, V_0 was the volume of titration by standard hydrochloric acid during blank calibration (mL), while V represented the volume of titration by standard hydrochloric acid during samples (mL). C_{HCl} was the concentration of standard hydrochloric acid (1 mol L^{-1}), m was soil mass (g), and a was soil water content (%).

The CO_2 efflux rate (mg CO_2 kg_{soil}^{-1} d^{-1}) was calculated with the following formula:

CO_2 efflux rate = CO_2 emission/t.

In this formula, t represented the day when the NaOH solution was placed in the incubation tanks.

The cumulative CO_2 emission (g CO_2 kg_{soil}^{-1}) was the cumulative CO_2 emission from each treatment over a given incubation time.

For a given incubation time (80 days), the priming index (PI) induced by exogenous substance addition was normalized to the proportion of added non-exogenous substances to cumulative CO_2 emissions based on the following formula:

Priming index (PI) = ($CO_{2\ add} - CO_{2\ non\text{-}add}$)/ $CO_{2\ non\text{-}add}$.

In this formula, $CO_{2\ add}$ represented cumulative CO_2 emissions after 80 days of defoliation and nitrogen treatment, and $CO_{2\ non\text{-}add}$ indicated cumulative CO_2 emissions after 80 days of treatment without defoliation and nitrogen.

The PI represented the intensity of the priming effect. The PI value of 1 suggested that the amount of organic carbon mineralization was not affected by exogenous substance addition. The PI value of >1 indicated that the addition of exogenous substances caused the priming effect of organic carbon mineralization, and the larger value was associated with a stronger priming effect. The PI value of <1 represented that the addition of exogenous substances reduced the mineralization of organic carbon and produced a negative priming effect, and the smaller value illustrated the stronger negative priming effect.

Statistical analysis

All statistical analyses were performed with the SPSS 18.0 statistical and R programming software. The effects of the CO_2 efflux rate, cumulative CO_2 emission, PI, MBC, catalase, alkaline phosphatase, cellulase, Chao1, Shannon, and Simpson and the relative abundance of *Proteobacteria* and *Acidobacteria* were analyzed with three-way analysis of variance combined with Duncan's multiple range test. Partial least squares discrimination analysis was conducted to analyze the distribution of soil microbial communities in soils at different depths. The Spearman correlation analysis was used to clarify the correlations of defoliation, nitrogen, and soil depth with CO_2 emissions, soil microbial activity parameters,

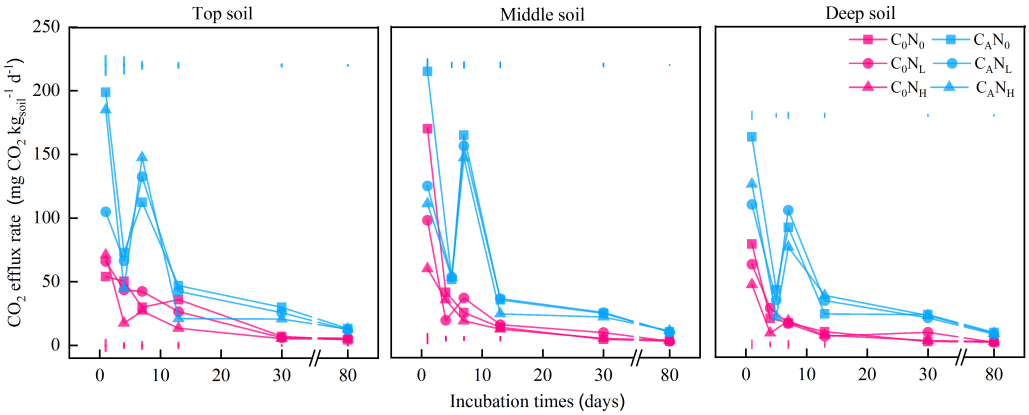

Figure 1 **CO_2 efflux rate (mg CO_2 $kg_{soil}^{-1}d^{-1}$) from the top soil, middle soil, and deep soil in each treatment over the entire incubation period.** C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input. The different colored bars show least significant differences (at 5% level) between nitrogen input levels within same defoliation addition at each sampling point.

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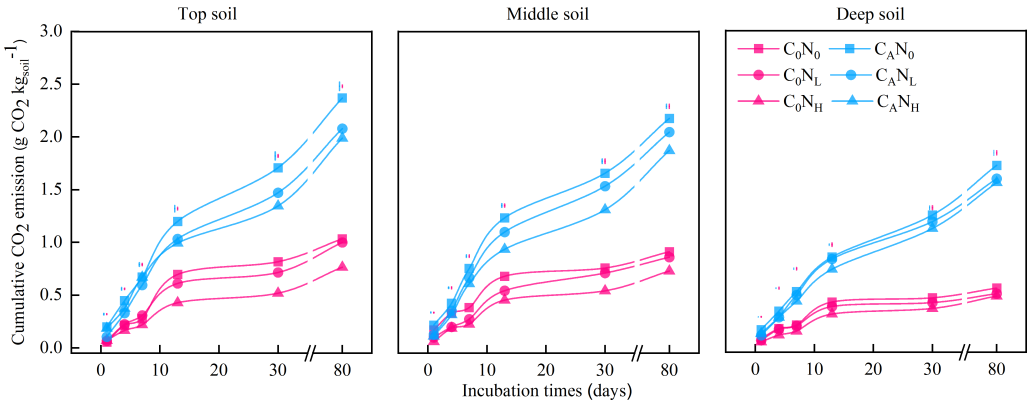

Figure 2 **Cumulative CO_2 emission (g CO_2 kg_{soil}^{-1}) from the top soil, middle soil, and deep soil in each treatment over the entire incubation period.** C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input. The different colored bars show least significant differences (at 5% level) between nitrogen input levels within same defoliation addition at each sampling point.

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Proteobacteria and *Acidobacteria*. Significance was defined at $P < 0.05$. Additionally, Origin18.0 and R programming software were utilized for mapping.

RESULTS

CO_2 emissions

The CO_2 efflux rate and cumulative CO_2 emissions were increased under defoliation addition, which was extremely significantly affected by the soil depth, defoliation, and nitrogen, as well as interaction among these three factors (Figs. 1 and 2, and Table 1).

Table 1 Statistical significance (*P* value) of the soil depth, defoliation addition, nitrogen input level, and their interaction on the concerned variables.

	Soil depth (S)	Defoliation (C)	Nitrogen (N)	S × C	S × N	C × N	S × C × N
CO_2 efflux rate (mg CO_2 kg_{soil}^{-1} d^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cumulative CO_2 emission (g CO_2 kg_{soil}^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PI index (80 days)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
MBC (4th day)	<0.001	<0.001	<0.001	<0.001	0.096	<0.001	0.01
Catalase (4th day)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Alkaline phosphatase (4th day)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cellulase (4th day)	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.07
Chao1 index (4th day)	<0.001	0.14	0.80	0.97	0.22	0.21	0.14
Shannon (4th day)	0.016	0.204	0.090	0.787	0.003	0.330	0.450
Simpson (4th day)	0.337	0.507	0.406	0.446	0.073	0.363	0.628
Relative abundance of Proteobacteria (%)	<0.001	<0.001	<0.001	<0.001	0.001	0.05	0.003
Relative abundance of Acidobacteria (%)	<0.001	0.052	<0.001	<0.001	<0.001	0.001	<0.001

The CO_2 efflux rate peaked on the 1st day and then declined with the incubation time of each treatment (Fig. 1). Among all treatments, the CO_2 efflux rate was higher in C_A treatment (65.41 mg CO_2 kg_{soil}^{-1} d^{-1}) than in C_0 treatment (26.42 mg CO_2 kg_{soil}^{-1} d^{-1}) and lower in deep soils (36.1 mg CO_2 kg_{soil}^{-1} d^{-1}) than middle (51.56 mg CO_2 kg_{soil}^{-1} d^{-1}) and top (50.06 mg CO_2 kg_{soil}^{-1} d^{-1}) soils (Fig. 1). In addition, N_0 treatment (52.87 mg CO_2 kg_{soil}^{-1} d^{-1}) resulted in a higher CO_2 efflux rate than N_L treatment (51.56 mg CO_2 kg_{soil}^{-1} d^{-1}) and N_H (50.06 mg CO_2 kg_{soil}^{-1} d^{-1}) on the 1st day.

After 80 days (Fig. 2), cumulative CO_2 emissions were averagely elevated by 2.62, 2.37, or 2.26 times under treatments of C_AN_0, C_AN_L, or C_AN_H when compared to under C_0N_0 treatment. Cumulative CO_2 emissions were 30.28% and 28.21% higher in top and middle soils than in deep soils, respectively. Meanwhile, cumulative CO_2 emissions were markedly lower under N_L and N_H treatment than under N_0 treatment by 7.87% and 15.75%, respectively.

PI

After 80 days of incubation, defoliation addition alone or in combination with nitrogen addition resulted in positive PI in soils at the three depths (Fig. 3). PI was extremely substantially affected by defoliation, nitrogen, and soil depth (Table 1). PI was the highest in deep soils (2.05 [C_AN_0], 1.83 [C_AN_L], and 1.76 [C_AN_H]) in defoliation and nitrogen additions. Moreover, nitrogen addition alone caused reverse PI in soils at three depths.

MBC

MBC was enhanced under defoliation and nitrogen addition, which was significantly or extremely significantly altered by defoliation, nitrogen and soil depth (Fig. 4 and Table 1). Moreover, defoliation addition alone or combined with nitrogen addition contributed to higher MBC than C_0N_0 treatment. On the 4th day, MBC in top soils was markedly higher under C_AN_0, C_AN_L, and C_AN_H treatment than under C_0N_0 treatment by 66.49%, 87.82%, and 97.81%. In contrast, nitrogen addition alone diminished MBC in soils at the three

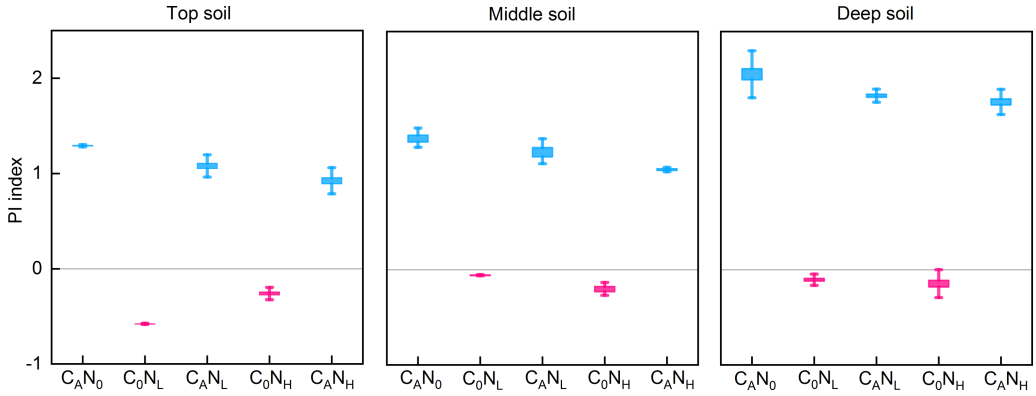

Figure 3 PI index from the top soil , middle soil, and deep soil in each treatment after 80days incubation. C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input.

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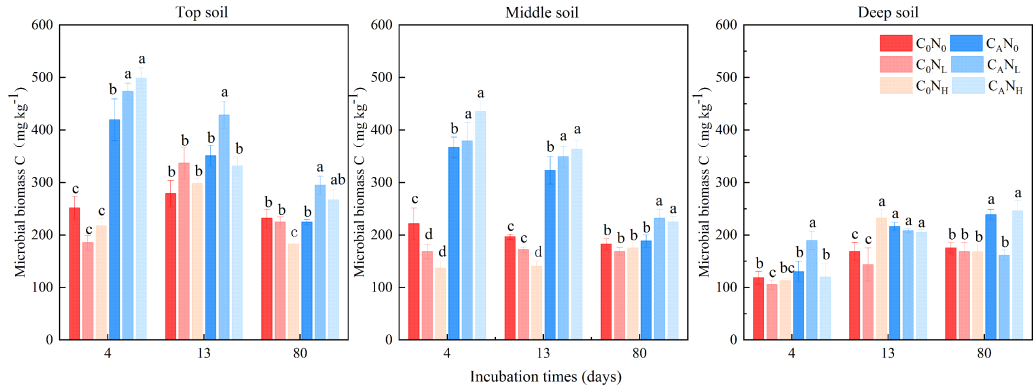

Figure 4 Soil microbial biomass C(MBC) at 4th, 13th, and 80th days after the start of the incubation in the top soil, middle soil, and deep soil. C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input. Different lowercase letters over the bar indicate significant difference at $p \leqq 0.05$ in the same incubation time.

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depths. MBC in top and middle soils was higher than that in deep soils by 127.07% and 104.31%, respectively.

Soil enzymes

The activity of soil catalase, alkaline phosphatase, and cellulase was enhanced, which was markedly changed by defoliation, nitrogen and soil depth, on the 4th day (Fig. 5 and Table 1). The activity of soil catalase was 32.95% and 36.94% higher, that of alkaline phosphatase was 84.64% and 83.03% higher, and that of cellulase was 174.67% and 251.12% higher under C_AN_L and C_AN_H treatment than under C_0N_0. Nitrogen addition alone had different influences on activity of the tested soil enzymes in soils at the three depths. The activity of catalase, alkaline phosphatase, and cellulase was higher in top soils than in deep soils.

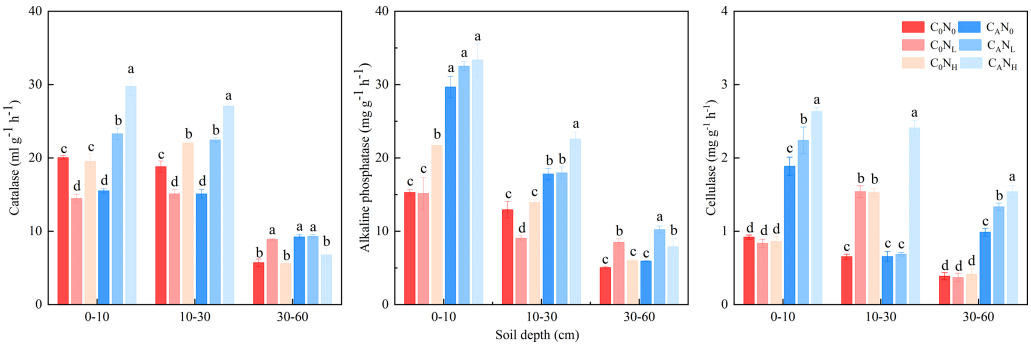

Figure 5 **Soil enzyme activity on 4th day in the top soil, middle soil, and deep soil.** C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input. Different lowercase letters over the bar indicate significant difference at $p \leqq 0.05$ in the same soil depth.

Full-size DOI: 10.7717/peerj.15276/fig-5

Soil bacterial diversity and community structure

During the first 7 days, CO_2 emissions rapidly surged after exogenous carbon addition. Mounting studies (*Tian et al., 2016a*; *Shen et al., 2021*; *Zhou et al., 2021*) reported that bacterial communities were changed from day 3 to day 15. Moreover, our study demonstrated that MBC peaked on the 4th day under defoliation and nitrogen addition. Accordingly, in order to evaluate the effect of defoliation, nitrogen, and soil depth on the soil microbial community structure in dryland cherry orchards, soil DNA was extracted on the 4th day of incubation to analyze the bacterial diversity. The results manifested that defoliation and nitrogen addition exerted no significant effects on the Chao1, Shannon, and Simpson index of soil bacteria (Fig. 6). However, the relative abundance of *Proteobacteria* markedly increased in soils at the three depths under defoliation and nitrogen addition, and that of *Acidobacteria* in deep soils was substantially reduced under both defoliation and nitrogen addition.

In our research, 48 phyla, 133 classes, 209 orders, 260 families, and 480 genera of bacteria were obtained through high-throughput sequencing of bacterial 16S rRNA. At the level of bacteria phyla (Fig. 7), the relative abundance of *Proteobacteria* and *Acidobacteria* was greater than 10.0% in communities, which were 37.84%–60.62% and 10.70%–24.53%, respectively. The relative abundance of *Proteobacteria* was 15.91% higher under C_A treatment than under C_0 treatment and was 12.18% and 11.85% higher under N_H and N_L treatment than under N_0 treatment in deep soils. The relative abundance of *Acidobacteria* in deep soils was lower under C_AN_H treatment than under C_0N_0 treatment by 96.23%.

DISCUSSION

Effects of defoliation and nitrogen addition on CO_2 emissions in soils at different depths

As previously described (*Fontaine, Mariotti & Abbadie, 2003*), defoliation alone or both defoliation and nitrogen was added in our study to assess organsim matter decomposition and analyze the mechanism of "microbial mining". Our data unveiled that cumulative

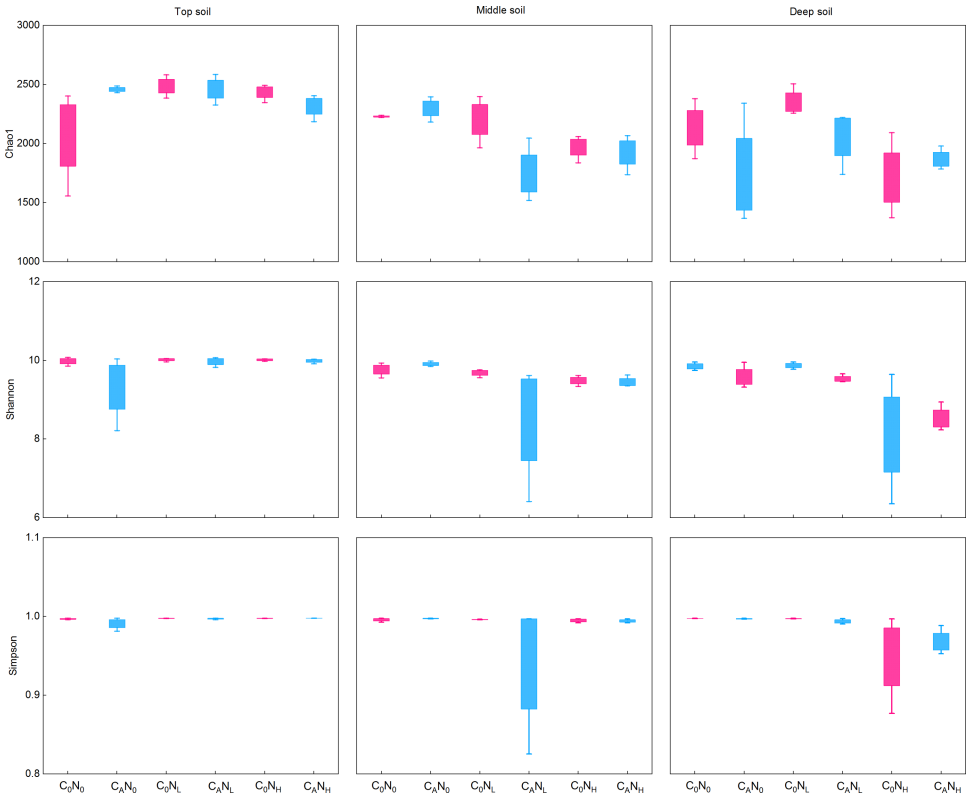

Figure 6 Richness (Chao1) and diversity (Shannon and Simpson) of bacteria in the top, middle, deep soils on 4th day. C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input.

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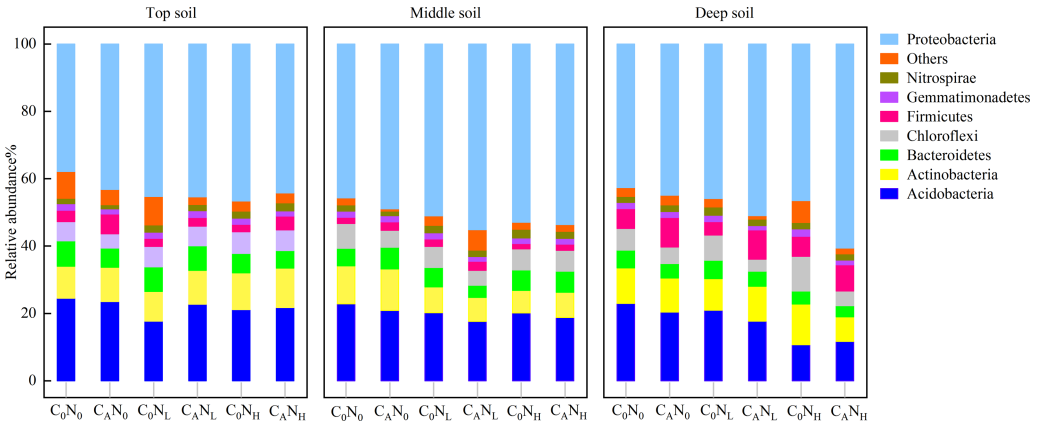

Figure 7 Relative abundance of bacteria phyla in the top, middle, deep soil on 4th day. C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input.

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CO_2 emissions under defoliation addition alone or combined with nitrogen addition were 1.94−2.78 times higher than those under C_0N_0 treatment, which may be attributable to

the fact that defoliation and nitrogen addition increased the activity of soil microbes (Figs. 4 and 5), accelerated the conversion of microbial biomass (*Blagodatskaya & Kuzyakov, 2008*), and facilitated the secretion of extracellular enzymes to decompose soil organsim matter and then deciduous residues through microbes (*Allison et al., 2010*; *Burns et al., 2013*), thus resulting in CO_2 emissions in short-term incubation (*Cotrufo et al., 2015*; *Liu et al., 2017*; *Zhou et al., 2021*). These results are consistent with hypothesis (1). Additionally, our findings also elucidated that compared to defoliation addition alone, addition of both defoliation and nitrogen decreased CO_2 emissions and PI, which is ascribed to the "mining of nitrogen" (*Fontaine, Mariotti & Abbadie, 2003*). Moreover, nitrogen alone addition also reduced CO_2 emissions and caused negative PI, which is attributed to the "mining of nitrogen" (*Fontaine, Mariotti & Abbadie, 2003*). Our data also revealed that in soils with relatively low total nitrogen contents (0.47−0.74 g kg^{-1}), microbes were stimulated to decompose organsim matter to acquire the required nitrogen and induce CO_2 emissions under defoliation and nitrogen addition, similar to most research results (*Chen et al., 2014*; *Li et al., 2017*; *Fang et al., 2018a*; *Mason-Jones, Schmücker & Kuzyakov, 2018*; *Hicks et al., 2019*; *Liao, Tian & Liu, 2021*). However, our data showed no significant differences between low and high nitrogen, which indicated that the nitrogen demand of microbes could be saturated at low nitrogen levels. In addition, nitrogen addition alone reduced CO_2 emissions and then caused negative PI, which was concordant with the results of laboratory culture in the dark (*Wang et al., 2014*; *Fang et al., 2018a*) and outdoor testing (*Ginting et al., 2003*). Corresponding to the research with the DeNitrification-DeComposition (DNDC) model (*Chi & Chen, 2001*), *Grant et al. (2004)* also used the DNDC model to predict the impact of 50% CO_2 emissions on nitrogen. Therefore, nitrogen addition alone exerts little effect on soil respiration, whilst combination of nitrogen and organic carbon effectively promotes the decomposition of exogenous organic substances.

Soil depth is frequently associated with new increases in CO_2 emissions (*Meyer et al., 2018*; *Liao et al., 2020*). Throughout the incubation period, the CO_2 efflux rates and cumulative CO_2 emissions were higher in top and middle soils than in deep soils, which might be explained by the following factors: (i) high MBC and activities of soil catalase, alkaline phosphatase, and cellulase in top and middle soils (Figs. 4 and 5), (ii) relatively sufficient nutrients in top and middle soils, and (iii) suitable pH and favorable soil structure and properties in top and middle soils (*Wang et al., 2014*; *Tian et al., 2016b*), which provided advantageous conditions for the decomposition of deciduous residues by microbes. Conversely, PI was higher in deep soils than in top and middle soils in our study, concurrent with the result of a prior study (*Liao et al., 2020*). The proportion of residual organic carbon was calculated based on the input amount of organic carbon and emission amount of CO_2 in soils at different depths, which exhibited that the proportion of residual organic carbon was 88.88%, 88.05%, and 80.60% in top, middle, and deep soils, respectively. This result indicated that top and middle soils can retain a higher proportion of exogenous carbon than deep soils.

Effects of defoliation and nitrogen addition on microbial activities and communities in different soils

Microbes have been extensively recognized to play a key role in soil carbon mineralization (*Herrmann & Bucksch, 2014*; *Chen et al., 2018*; *Li et al., 2018*). MBC and enzyme activities are usually considered measurable proxies for microbial decomposition (*Dorodnikov et al., 2009*; *Jiang et al., 2021*) and can be optimized with the shortest incubation time to minimize microbial growth and enzyme production during the measurement (*Shen et al., 2021*). Defoliation addition alone or combined with nitrogen addition elevated MBC, increased the activity of alkaline phosphatase, and cellulase, and caused positive PI in soils (Fig. 3). Defoliation shared significantly positive correlations with MBC and the activity of catalase, alkaline phosphatase, and cellulase in soils (Fig. 8). These data illustrated that microbes obtained available carbon and nitrogen from defoliation and nitrogen through various enzymes to meet their stoichiometric requirements (*Lashermes et al., 2016*). In our study, nitrogen increased microbial biomass and the activity of the tested enzymes in defoliation and nitrogen additions (Figs. 4 and 5) as a regulator in organic carbon mineralization, which was supported by many previous studies (*Frey et al., 2004*; *Cayuela, Sinicco & Mondini, 2009*; *Parajuli, Ye & Szogi, 2022*). We also observed that at the initial stage of culture, addition of both defoliation and nitrogen (especially both defoliation and high nitrogen) markedly enhanced MBC and enzyme activities but diminished CO_2 emissions when compared with defoliation addition alone, further confirming the "microbial mining" mechanism (*Fontaine, Mariotti & Abbadie, 2003*). Meanwhile, our results also unraveled that only nitrogen addition significantly decreased soil microbial biomass and changed the activity of the tested enzymes at the three depths, accompanied by reduced CO_2 emissions and negative PI. These findings illustrated that the effective combination of nitrogen and organic carbon could accelerate organism decomposition, improve soil quality and fertility, and provide guidance for production practice.

In our study, *Proteobacteria* and *Acidobacteria* were dominant bacteria in soils regardless of soil depth or exogenous inputs (Fig. 7). Defoliation and nitrogen addition did not significantly change soil bacterial diversity and abundance (Fig. 6), indicating the ecological properties of *Proteobacteria* and *Acidobacteria* in soils. Defoliation addition alone or combined with nitrogen addition markedly elevated the relative abundance of *Proteobacteria* and reduced the relative abundance of *Acidobacteria*, thereby causing high CO_2 emissions. This result suggested the association of *Proteobacteria* and *Acidobacteria* with CO_2 emissions. As a copiotrophic group, *Proteobacteria* grows rapidly, and its abundance is increased by relying on more labile carbon sources under nutrient inputs (*Ramirez, Craine & Fierer, 2012*). With increasing stimulation, more *Proteobacteria* can participate in the synthesis of exoenzymes to mineralize substrates (*Schimel, 2003*; *Fierer, Bradford & Jackson, 2007*), corresponding to the result that defoliation addition alone or combined with nitrogen addition substantially augmented MBC and enzyme activities, which was consistent with hypothesis (2). Our data revealed that CO_2 emissions were significantly positively correlated with *Proteobacteria* (Fig. 8). In addition, *Nottingham et al. (2009)* observed that microbial biomass increased in the first step of plant residue decomposition, which was caused by labile C rather than macromolecular compounds.

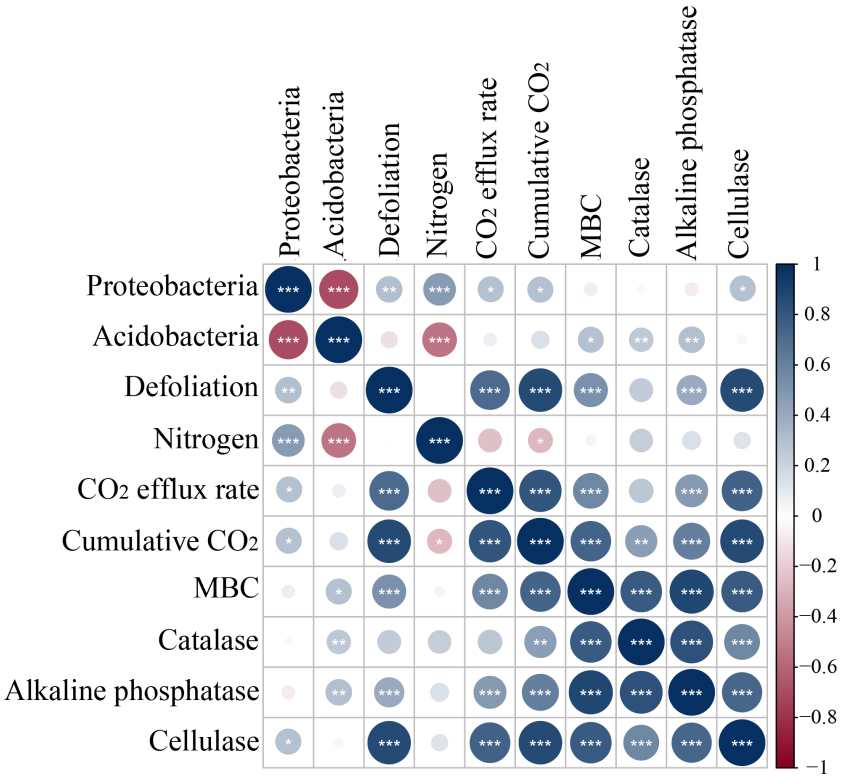

Figure 8 Spearman correlation relationship among the relative abundance of bacteria symbols coded on 4th day. Red negative; Blue positive. Darker colors indicate stronger correlationships, asterisks indicate significant effects (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).

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Altogether, these data suggest *Proteobacteria* as a main participant in organic carbon mineralization in cherry orchards. However, *Acidobacteria* (an oligotrophic group) could be colonized on mineral surfaces under rich nutrient conditions (*Nemergut et al., 2010*; *Ramirez, Craine & Fierer, 2012*).

Furthermore, our PLS-DA analysis (Fig. 9) demonstrated that bacterial communities were clearly separated in top and deep soils, which can be explained by the fact that nutrient composition determines the distribution of oligotrophic and hypertrophic bacteria (*Fierer, Bradford & Jackson, 2007*; *Whitman et al., 2016*), consistent with hypothesis (3). Compared to top soils, deep soils had the highest relative abundance of *Proteobacteria* under both defoliation and nitrogen addition, which can be attributed to the highest PI in deep soils that is related to different microbial products (*Kögel-Knabner, 2017*), soil pH (*Madsen & Munk, 1987*; *Silveira et al., 2008*; *Takele, Chimdi & Abebaw, 2014*), and soil nutrient (*Madsen & Munk, 1987*). Therefore, soil microbial communities may be partially determined by soil properties. Furthermore, fungi have also been reported to directly participate in organic carbon mineralization. Accordingly, further research is warranted to determine how fungal communities respond to defoliation and nitrogen added in soils of cherry orchards in northwest China.

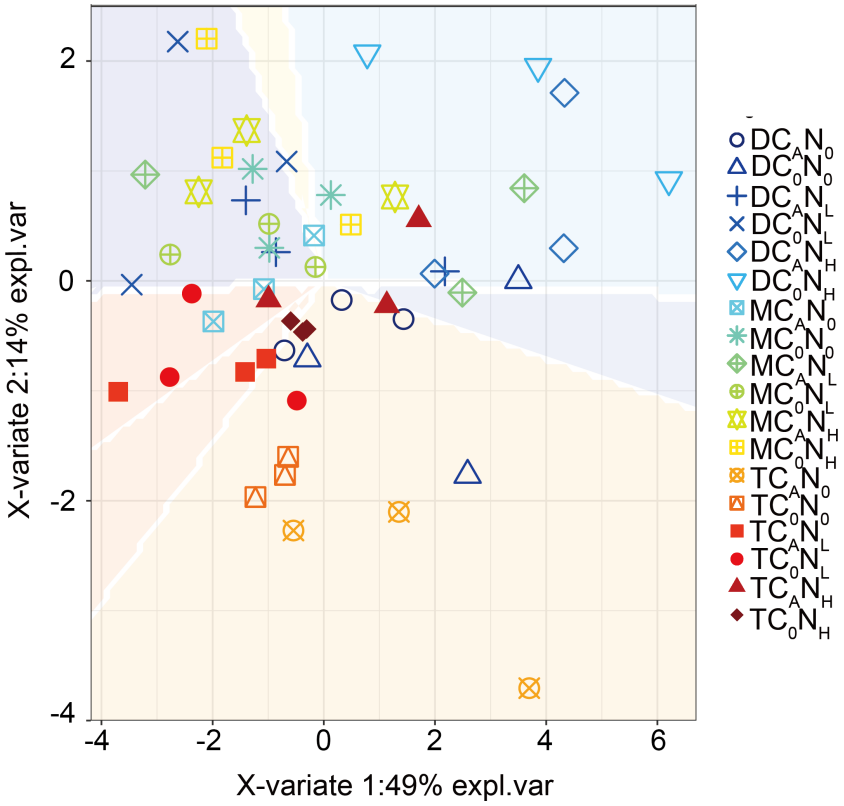

Figure 9 **PLS$_{DA}$ of the bacterial communities on 4th day.** T, top soil; M, middle soil; D, deep soil; C_0, no-defoliation addition; C_A, defoliation addition; N_0, no-nitrogen input; N_L, low-nitrogen input; N_H, high-nitrogen input.

Full-size DOI: 10.7717/peerj.15276/fig-9

CONCLUSION

In the early stages of the incubation period, defoliation and nitrogen addition increased MBC, the activity of catalase, alkaline phosphatase, and cellulase, and CO_2 emissions and resulted in positive PI in soils at the three depths of dryland cherry orchards. Nitrogen addition alone contributed to a significant reduction in MBC and CO_2 emissions and negative PI. Meanwhile, defoliation and nitrogen addition markedly elevated the relative abundance of *Proteobacteria* and reduced the relative abundance of *Acidobacteria* in soils at the three depths, particularly deep soils. Moreover, *Proteobacteria* was significantly correlated with defoliation, nitrogen, CO_2 emissions, and soil cellulase enzyme activities. In conclusion, this study unraveled the separate and interactive effects of defoliation and nitrogen addition on CO_2 emissions, soil microbial activities, and soil microbial communities composition at the soil depth scale. Likewise, this study demonstrated that both defoliation and nitrogen addition simulated exogenous carbon decomposition, affected soil microbial communities and improved soil microbial activities, ultimately enhancing soil quality in dryland cherry orchards.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Jing Wang conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yibo Wang conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Ruifang Xue conceived and designed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Dandan Wang conceived and designed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Wenhui Nan conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The data is available at NCBI SRA: PRJNA896137.

Supplemental Information

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REFERENCES

Allison SD, Weintraub MN, Gartner TB, Waldrop MP. 2010. Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. 229–243 DOI 10.1007/978-3-642-14225-3_12.

Aye NS, Butterly CR, Sale PWG, Tang C. 2018. Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocellulose. *Soil Biology and Biochemistry* **123**:33–44 DOI 10.1016/j.soilbio.2018.04.027.

Banfield CC, Pausch J, Kuzyakov Y, Dippold MA. 2018. Microbial processing of plant residues in the subsoil—the role of biopores. *Soil Biology and Biochemistry* **125**:309–318 DOI 10.1016/j.soilbio.2018.08.004.

Blagodatskaya EV, Blagodatsky SA, Anderson T-H, Kuzyakov Y. 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology* **37**:95–105 DOI 10.1016/j.apsoil.2007.05.002.

Blagodatskaya E, Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* **45**:115–131 DOI 10.1007/s00374-008-0334-y.

Blanco-Canqui H, Lal R. 2008. No-tillage and soil-profile carbon sequestration: an on-farm assessment. *Soil Science Society of America Journal* **72**:693–701 DOI 10.2136/sssaj2007.0233.

Bond-Lamberty B, Bailey VL, Chen M, Gough CM, Vargas R. 2018. Globally rising soil heterotrophic respiration over recent decades. *Nature* **560**:80–83 DOI 10.1038/s41586-018-0358-x.

Borrajo RH, Zandio M, Zarzuela A, Serrano JF, Peralta V, Cuesta MJ, Rosa A, Fañanás L. 2011. Validity of maternal recall of obstetric complications in mothers of patients with schizophrenia spectrum disorders and their healthy siblings. *Schizophrenia Research* **126**:308–309 DOI 10.1016/j.schres.2010.09.017.

Buchmann N. 2000. Biotic and abiotic factors controlling soil respiration rates in Picea abies stands. *Soil Biology and Biochemistry* **32**:1625–1635 DOI 10.1016/S0038-0717(00)00077-8.

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A. 2013. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry* **58**:216–234 DOI 10.1016/j.soilbio.2012.11.009.

Cayuela ML, Sinicco T, Mondini C. 2009. Mineralization dynamics and biochemical properties during initial decomposition of plant and animal residues in soil. *Applied Soil Ecology* **41**:118–127 DOI 10.1016/j.apsoil.2008.10.001.

Chen L, Liu L, Mao C, Qin S, Wang J, Liu F, Blagodatsky S, Yang G, Zhang Q, Zhang D, Yu J, Yang Y. 2018. Nitrogen availability regulates topsoil carbon dynamics after permafrost thaw by altering microbial metabolic efficiency. *Nature Communications* **9**:3951 DOI 10.1038/s41467-018-06232-y.

Chen R, Senbayram M, Blagodatsky S, Myachina O, Dittert K, Lin X, Blagodatskaya E, Kuzyakov Y. 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* **20**:2356–2367 DOI 10.1111/gcb.12475.

Chen L, Zhang J, Zhao B, Zhou G, Ruan L. 2015. Bacterial community structure in maize stubble-amended soils with different moisture levels estimated by bar-coded

pyrosequencing. *Applied Soil Ecology* **86**:62–70 DOI 10.1016/j.apsoil.2014.09.011.

Chi CY, Chen CH. 2001. MIMO inverse filter criteria and blind maximum ratio combining using HOS for equalization of DS/CDMA systems in multipath. In: *IEEE Work. Signal Process. Adv. Wirel. Commun. SPAWC 2001-Janua*. 114–117 DOI 10.1109/SPAWC.2001.923859.

Cotrufo MF, Soong JL, Horton AJ, Campbell EE, Haddix ML, Wall DH, Parton WJ. 2015. Formation of soil organic matter *via* biochemical and physical pathways of litter mass loss. *Nature Geoscience* **8**:776–779 DOI 10.1038/ngeo2520.

Craine JM, Morrow C, Fierer N. 2007. Microbial nitrogen limitation increases decomposition. *Ecology* **88**:2105–2113 DOI 10.1890/06-1847.1.

Cui J, Zhu Z, Xu X, Liu S, Jones DL, Kuzyakov Y, Shibistova O, Wu J, Ge T. 2020. Carbon and nitrogen recycling from microbial necromass to cope with C:N stoichiometric imbalance by priming. *Soil Biology and Biochemistry* **142**:107720 DOI 10.1016/j.soilbio.2020.107720.

De Graaff M-A, Jastrow JD, Gillette S, Johns A, Wullschleger SD. 2014. Differential priming of soil carbon driven by soil depth and root impacts on carbon availability. *Soil Biology and Biochemistry* **69**:147–156 DOI 10.1016/j.soilbio.2013.10.047.

Dorodnikov M, Blagodatskaya E, Blagodatsky S, Marhan S, Fangmeier A, Kuzyakov Y. 2009. Stimulation of microbial extracellular enzyme activities by elevated CO 2 depends on soil aggregate size. *Global Change Biology* **15**:1603–1614 DOI 10.1111/j.1365-2486.2009.01844.x.

Fang Y, Nazaries L, Singh BK, Singh BP. 2018a. Microbial mechanisms of carbon priming effects revealed during the interaction of crop residue and nutrient inputs in contrasting soils. *Global Change Biology* **24**:2775–2790 DOI 10.1111/gcb.14154.

Fang Y, Singh BP, Collins D, Li B, Zhu J, Tavakkoli E. 2018b. Nutrient supply enhanced wheat residue-carbon mineralization, microbial growth, and microbial carbon-use efficiency when residues were supplied at high rate in contrasting soils. *Soil Biology and Biochemistry* **126**:168–178 DOI 10.1016/j.soilbio.2018.09.003.

Fierer N, Bradford MA, Jackson RB. 2007. Toward an ecological classification of soil bacteria. *Ecology* **88**:1354–1364 DOI 10.1890/05-1839.

Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* **450**:277–280 DOI 10.1038/nature06275.

Fontaine S, Mariotti A, Abbadie L. 2003. The priming effect of organic matter: a question of microbial competition?. *Soil Biology and Biochemistry* **35**:837–843 DOI 10.1016/S0038-0717(03)00123-8.

Frey SD, Knorr M, Parrent JL, Simpson RT. 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management* **196**:159–171 DOI 10.1016/j.foreco.2004.03.018.

Ginting D, Kessavalou A, Eghball B, Doran JW. 2003. Greenhouse gas emissions and soil indicators four years after manure and compost applications. *Journal of Environmental Quality* **32**:23–32 DOI 10.2134/jeq2003.2300.

Grant B, Smith WN, Desjardins R, Lemke R, Li C. 2004. Estimated N2O and CO2 emissions as influenced by agricultural practices in Canada. *Climate Change* **65**:315–332 DOI 10.1023/B:CLIM.0000038226.60317.35.

Guan S, Zhang D, Zhang Z. 1986. *Soil enzyme and its research methods*. Beijing: Agriculture Press.

Herrmann H, Bucksch H. 2014. Soil respiration. In: *Dictionary geotechnical engineering/wörterbuch geotechnik*. Berlin, Heidelberg: Springer, 1268–1268 DOI 10.1007/978-3-642-41714-6_195345.

Hessen DO, Ågren GI, Anderson TR, Elser JJ, De Ruiter PC. 2004. Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology* **85**:1179–1192 DOI 10.1890/02-0251.

Hicks LC, Meir P, Nottingham AT, Reay DS, Stott AW, Salinas N, Whitaker J. 2019. Carbon and nitrogen inputs differentially affect priming of soil organic matter in tropical lowland and montane soils. *Soil Biology and Biochemistry* **129**:212–222 DOI 10.1016/j.soilbio.2018.10.015.

Jiang Z, Liu Y, Yang J, Brookes PC, Gunina A. 2021. Rhizosphere priming regulates soil organic carbon and nitrogen mineralization: the significance of abiotic mechanisms. *Geoderma* **385**:114877 DOI 10.1016/j.geoderma.2020.114877.

Kögel-Knabner I. 2017. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter: fourteen years on. *Soil Biology and Biochemistry* **105**:A3–A8 DOI 10.1016/j.soilbio.2016.08.011.

Lashermes G, Gainvors-Claisse A, Recous S, Bertrand I. 2016. Enzymatic strategies and carbon use efficiency of a litter-decomposing fungus grown on maize leaves, stems, and roots. *Frontiers in Microbiology* **7**:1315 DOI 10.3389/fmicb.2016.01315.

Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America* **112**:10967–10972 DOI 10.1073/pnas.1508382112.

Li L, Barker XZhu, Ye R, Doane TA, Horwath WR. 2018. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. *Soil Biology and Biochemistry* **119**:41–49 DOI 10.1016/j.soilbio.2018.01.003.

Li Q, Tian Y, Zhang X, Xu X, Wang H, Kuzyakov Y. 2017. Labile carbon and nitrogen additions affect soil organic matter decomposition more strongly than temperature. *Applied Soil Ecology* **114**:152–160 DOI 10.1016/j.apsoil.2017.01.009.

Liang Z, Cao B, Jiao Y, Liu C, Li X, Meng X, Shi J, Tian X. 2022. Effect of the combined addition of mineral nitrogen and crop residue on soil respiration, organic carbon

sequestration, and exogenous nitrogen in stable organic matter. *Applied Soil Ecology* **171**:104324 DOI 10.1016/j.apsoil.2021.104324.

Liao C, Li D, Huang L, Yue P, Liu F, Tian Q. 2020. Higher carbon sequestration potential and stability for deep soil compared to surface soil regardless of nitrogen addition in a subtropical forest. *PeerJ* **8**:e9128 DOI 10.7717/peerj.9128.

Liao C, Tian Q, Liu F. 2021. Nitrogen availability regulates deep soil priming effect by changing microbial metabolic efficiency in a subtropical forest. *Journal of Forestry Research* **32**:713–723 DOI 10.1007/s11676-020-01148-0.

Liu X-JA, Sun J, Mau RL, Finley BK, Compson ZG, Van Gestel N, Brown JR, Schwartz E, Dijkstra P, Hungate BA. 2017. Labile carbon input determines the direction and magnitude of the priming effect. *Applied Soil Ecology* **109**:7–13 DOI 10.1016/j.apsoil.2016.10.002.

Madsen HB, Munk I. 1987. The influence of texture, soil depth and geology on pH in farmland soils. *Acta Agriculturae Scandinavica B* **37**:407–418 DOI 10.1080/00015128709436572.

Malhi SS, Harapiak JT, Nyborg M, Gill KS, Monreal CM, Gregorich EG. 2003. Light fraction organic N, ammonium, nitrate and total N in a thin Black Chernozemic soil under bromegrass after 27 annual applications of different N rates. *Nutrient Cycling in Agroecosystems* **65**:201–210 DOI 10.1023/A:1022623405707.

Mason-Jones K, Schmücker N, Kuzyakov Y. 2018. Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming. *Soil Biology and Biochemistry* **124**:38–46 DOI 10.1016/j.soilbio.2018.05.024.

Meyer N, Welp G, Rodionov A, Borchard N, Martius C, Amelung W. 2018. Nitrogen and phosphorus supply controls soil organic carbon mineralization in tropical topsoil and subsoil. *Soil Biology and Biochemistry* **119**:152–161 DOI 10.1016/j.soilbio.2018.01.024.

Miralles I, Domingo F, Cantón Y, Trasar-Cepeda C, Leirós MC, Gil-Sotres F. 2012. Hydrolase enzyme activities in a successional gradient of biological soil crusts in arid and semi-arid zones. *Soil Biology and Biochemistry* **53**:124–132 DOI 10.1016/j.soilbio.2012.05.016.

Mo F, Zhang Y-Y, Liu Y, Liao Y-C. 2021. Microbial carbon-use efficiency and straw-induced priming effect within soil aggregates are regulated by tillage history and balanced nutrient supply. *Biology and Fertility of Soils* **57**:409–420 DOI 10.1007/s00374-021-01540-w.

Moorhead DL, Sinsabaugh RL, Moorhead L. 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs* **76**:151–174 DOI 10.1890/0012-9615(2006)076[0151:ATMOLD]2.0.CO;2.

Na M, Yuan M, Hicks LC, Rousk J. 2022. Testing the environmental controls of microbial nitrogen-mining induced by semi-continuous labile carbon additions in the subarctic. *Soil Biology and Biochemistry* **166**:108562 DOI 10.1016/j.soilbio.2022.108562.

Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD. 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* **419**:915–917 DOI 10.1038/nature01136.

Nemergut DR, Cleveland CC, Wieder WR, Washenberger CL, Townsend AR. 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. *Soil Biology and Biochemistry* **42**:2153–2160 DOI 10.1016/j.soilbio.2010.08.011.

Nottingham AT, Griffiths H, Chamberlain PM, Stott AW, Tanner EVJ. 2009. Soil priming by sugar and leaf-litter substrates: a link to microbial groups. *Applied Soil Ecology* **42**:183–190 DOI 10.1016/j.apsoil.2009.03.003.

Parajuli B, Ye R, Szogi A. 2022. Mineral N suppressed priming effect while increasing microbial C use efficiency and N2O production in sandy soils under long-term conservation management. *Biology and Fertility of Soils* **58**:903–915 DOI 10.1007/s00374-022-01665-6.

Raich JW, Tufekcioglu A. 2000. Vegetation and soil respiration: correlations and controls. *Biogeochemistry* **48**:71–90 DOI 10.1023/A:1006112000616.

Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* **18**:1918–1927 DOI 10.1111/j.1365-2486.2012.02639.x.

Razanamalala K, Fanomezana RA, Razafimbelo T, Chevallier T, Trap J, Blanchart E, Bernard L. 2018. The priming effect generated by stoichiometric decomposition and nutrient mining in cultivated tropical soils: actors and drivers. *Applied Soil Ecology* **126**:21–33 DOI 10.1016/j.apsoil.2018.02.008.

Sawada K, Inagaki Y, Toyota K. 2021. Priming effects induced by C and N additions in relation to microbial biomass turnover in Japanese forest soils. *Applied Soil Ecology* **162**:103884 DOI 10.1016/j.apsoil.2021.103884.

Schimel J. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* **35**:549–563 DOI 10.1016/S0038-0717(03)00015-4.

Shahbaz M, Kumar A, Kuzyakov Y, Börjesson G, Blagodatskaya E. 2018. Priming effects induced by glucose and decaying plant residues on SOM decomposition: a three-source 13C/14C partitioning study. *Soil Biology and Biochemistry* **121**:138–146 DOI 10.1016/j.soilbio.2018.03.004.

Shen Q, Redmile-Gordon M, Song J, Li J, Zhang K, Voroney P, Xu J, Brookes PC. 2021. Amendment with biodiesel co-product modifies genes for N cycling (nirK, nirS, nosZ) and greenhouse gas emissions (N2O, CH4, CO2) from an acid soil. *Biology and Fertility of Soils* **57**:629–642 DOI 10.1007/s00374-021-01546-4.

Silveira ML, Comerford NB, Reddy KR, Cooper WT, El-Rifai H. 2008. Characterization of soil organic carbon pools by acid hydrolysis. *Geoderma* **144**:405–414 DOI 10.1016/j.geoderma.2008.01.002.

Tabatabai MA, Bremner JM. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**:301–307 DOI 10.1016/0038-0717(69)90012-1.

Takele L, Chimdi A, Abebaw A. 2014. Dynamics of soil fertility as influenced by different land use systems and soil depth in west Showa Zone, Gindeberet district, Ethiopia. *Agriculture, Forestry and Fisheries* **3(6)**:489–494 DOI 10.11648/j.aff.20140306.18.

Tang X, Du J, Shi Y, Lei N, Chen G, Cao L, Pei X. 2020. Global patterns of soil heterotrophic respiration—a meta-analysis of available dataset. *Catena* **191**:104574 DOI 10.1016/j.catena.2020.104574.

Tian J, Pausch J, Yu G, Blagodatskaya E, Kuzyakov Y. 2016a. Aggregate size and glucose level affect priming sources: a three-source-partitioning study. *Soil Biology and Biochemistry* **97**:199–210 DOI 10.1016/j.soilbio.2016.03.013.

Tian Q, Yang X, Wang X, Liao C, Li Q, Wang M, Wu Y, Liu F. 2016b. Microbial community mediated response of organic carbon mineralization to labile carbon and nitrogen addition in topsoil and subsoil. *Biogeochemistry* **128**:125–139 DOI 10.1007/s10533-016-0198-4.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* **19**:703–707 DOI 10.1016/0038-0717(87)90052-6.

Wang C, Liu D, Bai E. 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biology and Biochemistry* **120**:126–133 DOI 10.1016/j.soilbio.2018.02.003.

Wang J, Song B, Ma F, Tian D, Li Y, Yan T, Quan Q, Zhang F, Li Z, Wang B, Gao Q, Chen W, Niu S. 2019. Nitrogen addition reduces soil respiration but increases the relative contribution of heterotrophic component in an alpine meadow. *Functional Ecology* **33**:2239–2253 DOI 10.1111/1365-2435.13433.

Wang Q, Wang S, He T, Liu L, Wu J. 2014. Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils. *Soil Biology and Biochemistry* **71**:13–20 DOI 10.1016/j.soilbio.2014.01.004.

Whitman T, Pepe-Ranney C, Enders A, Koechli C, Campbell A, Buckley DH, Lehmann J. 2016. Dynamics of microbial community composition and soil organic carbon mineralization in soil following addition of pyrogenic and fresh organic matter. *The ISME Journal* **10**:2918–2930 DOI 10.1038/ismej.2016.68.

Xu G, Zheng Y. 1986. *Handbook of analysis of soil microorganism*. Beijing: China Agriculture Press.

Zhou W, Qin X, Lyu D, Qin S. 2021. Effect of glucose on the soil bacterial diversity and function in the rhizosphere of Cerasus sachalinensis. *Horticultural Plant Journal* **7**:307–317 DOI 10.1016/j.hpj.2021.02.002.