Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO₂

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Increased plant productivity and decreased microbial respiratory C loss can potentially mitigate increasing atmospheric CO₂, but we currently lack effective means to achieve these goals. Soil microbes may play critical roles in mediating plant productivity and soil C/N dynamics under future climate scenarios of elevated CO₂ (eCO₂) through optimizing functioning of the root-soil interface. By using a labeling technique with ¹³C and ¹⁵N, we examined the effects of plant growth-promoting Pseudomonas fluorescens on C and N cycling in the rhizosphere of a common grass species under eCO₂. These microbial inoculants were shown to increase plant productivity. Although strong competition for N between the plant and soil microbes was observed, the plant can increase its capacity to store more biomass C per unit of N under P. fluorescens addition. Unlike eCO₂ effects, P. fluorescens inoculants did not change mass-specific microbial respiration and accelerate soil decomposition related to N cycling, suggesting these microbial inoculants mitigated positive feedbacks of soil microbial decomposition to eCO₂. The potential to mitigate climate change by optimizing soil microbial functioning by plant growth-promoting Pseudomonas fluorescens is a prospect for ecosystem management.

Increased plant productivity and decreased microbial respiratory C loss could potentially mitigate increasing atmospheric CO₂ concentrations, but we currently lack effective means to achieve these goals¹²-³. The accumulation of ecosystem C is controlled by the balance between plant productivity versus heterotrophic respiration through soil organic matter (SOM) decomposition⁴-⁶. Numerous studies have reported that elevated CO₂ (eCO₂) promotes plant growth and increases photosynthetic C input to soils⁷-⁸. These increased C inputs can stimulate microbial growth and N demand, which can limit soil N availability and plant N uptake under eCO₂⁹. On the other hand, positive feedbacks of soil microbial communities under eCO₂ may accelerate SOM decomposition and potentially result in soil net C losses¹¹-¹³. It is clear that microbial dynamics play an important role in regulating net ecosystem C storage under future climates, but potentially counteracting responses make it difficult to determine their net effect.

Some beneficial microbial inoculants have been shown to increase plant growth by improving soil N availability in many managed ecosystems under ambient CO₂¹⁴-¹⁶. However, stoichiometric homeostasis theory suggests that increased plant C fixation under eCO₂ can accelerate soil decomposition related to N mineralization by increasing the allocation of resources towards the microbial production of enzymes that degrade N-rich substrates¹⁷-¹⁹. Recent studies suggest that eCO₂ promotes SOM decomposition associated with microbial activity through increased rhizosphere priming effects (RPEs)²⁰-²¹. Increased microbial utilization of C exudate under eCO₂ can induce increased N mineralization and create positive nutrient feedbacks to mitigate soil N limitation²¹-²³. However, in order to increase the efficiency by which microbes metabolize and transform plant detritus under eCO₂, microbial inoculants should increase plant productivity, without accelerating SOM decomposition rates and microbial respiratory C loss. For example, applications of arbuscular mycorrhizal fungi (AMF) were generally shown to facilitate plant growth and stimulate soil carbon storage under ambient CO₂²⁴-²⁵. However, AMF can
speed up the turnover of recently fixed photosynthetic C associated with accelerated N cycling in spite of increased plant net primary productivity in forests exposed to eCO2, limiting soil C accumulation20,21.

Many isolated strains have been identified as plant growth-promoting rhizobacteria (PGPR), which could be used to stimulate plant growth under rising atmospheric CO226,27. Numerous studies have shown that PGPR can effectively increase plant performance in nutrient-limited environments, through positively influencing root growth and morphology, and promoting other beneficial plant–microbe symbioses28,29. In various natural terrestrial ecosystems, application of PGPR inoculants has emerged as a technology to facilitate grassland management30, ecosystem restoration27 and reforestation31. In contrast to soil saprotrophic bacteria, PGPR appear to have high substrate use efficiencies32. Under future climate conditions, eCO2 may increase PGPR dominance as these plant-associated microbes enhance plant success under eCO226,33. Nevertheless, our understanding of how plants interact with PGPR inoculants to regulate SOM decomposition and soil N availability in the rhizosphere is still limited, despite the potential of PGPB to alleviate environmental stresses imposed by eCO226,27. The effective application of these PGPB in natural terrestrial ecosystems requires understanding the traits that may enhance ecological performance in the rhizosphere3,16.

Here, we used a dual-isotope labeling technique with 13C and 15N to evaluate the effect of Pseudomonas fluorescens inoculation on plant productivity and soil C/N cycling under eCO2. P. fluorescens, which is common in soils, improves plant growth through several different mechanisms, such as the suppression of plant diseases and enhancement of essential metal uptake15,28,31. The objectives of the current study were to test whether using P. fluorescens as a soil microbial inoculum (1) improves plant productivity and photosynthetic C input to soils, (2) promotes plant N use, and (3) mitigates C lost through microbial respiration.

Results

Bacteria and eCO2 had positive, cumulative effects on plant productivity in terms of total plant biomass C (Figure 1a). The highest plant productivity occurred in the combined bacteria and eCO2 treatment (Figure 1a). In addition, bacteria and eCO2 increased the plant C:N ratio as individual treatments, but demonstrated a synergistic effect when combined (Figure 1b), suggesting that plants can assimilate more C per unit of N in tissue under eCO2 with these P. fluorescens inoculants. Increased plant root surface area associated with bacterial inoculants and eCO2 (Table S1) demonstrated a strong positive relationship with plant tissue C:N (Figure 2a). Combined bacterial inoculant and eCO2 treatments demonstrated significantly lower total soil enzyme C:N acquisition activity ratios (Table S1); and decreases in enzyme C:N were strongly correlated with higher plant tissue C:N (Figure 2b), suggesting a strong competition for N between plant and soil microbes.

We took advantage of the distinct δ15N values to estimate the relative sources of plant N (N from the original inorganic pool vs. N mineralized from SOM). eCO2 significantly increased the δ15N value in plant biomass (Figure 3a). The higher δ15N in soil organic N compared to soil inorganic N (see δ15N values of two pools in Methods) indicates that eCO2 increased plant uptake of mineralized N from SOM relative to soil inorganic N. However, P. fluorescens inoculants did not change plant δ15N values at each CO2 level.

Figure 1 | Plant C pool size per pot (a) and biomass C:N ratio (b). Control: ambient CO2 and without bacteria addition; B: ambient CO2 and with bacteria addition; eCO2: elevated CO2 and without bacteria addition; B + eCO2: elevated CO2 and with bacteria addition. Error bars show standard error of the mean (n = 6). The same letters denote non-significant differences between treatments (P > 0.05).

Figure 2 | Linear relationships of plant C:N ratio with root surface area (a) and total C:N enzyme ratio (b) across all treatments. Control: ambient CO2 and without bacteria addition; B: ambient CO2 and with bacteria addition; eCO2: elevated CO2 and without bacteria addition; B + eCO2: elevated CO2 and with bacteria addition.
root length (c) across all treatments.

Although high variability in rhizosphere priming effects was observed among the treatments, priming of SOM decomposition was positively related with plant δ15N (Figure 3b), suggesting that rhizosphere exudation (priming) was induced by plants in order to facilitate N mineralization from SOM. Likewise, total root length (which determines root system expansion) was positively related with plant δ15N (Figure 3c).

Compared to the control, all treatments induced higher soil C inputs from the plant-derived C (Figure 4a). However, significantly higher mass-specific microbial respiration (lower C use efficiency) was observed only under eCO2 (Figure 4b).

Discussion

The capability of plants and soil microbes to successfully sequester atmospheric C in terrestrial ecosystems largely depends on plant productivity along with microbial decomposition and mineralization feedbacks within the rhizosphere.8,10–12. Advancing current efforts to mitigate effects of climate change could minimize harmful effects of elevated CO2.1,26. Here, for the first time, we show that addition of a microbial inoculant has the potential to promote plant productivity while mitigating positive feedbacks of microbial decomposition to increased plant C inputs that typically accompany eCO2. Our results also demonstrated that P. fluorescens inoculation led to increased plant tissue C:N under eCO2, resulting in an increased capacity to store C per unit of N in plant tissue. Therefore, this soil microbial inoculant may be a useful tool to mitigate climate change.

The results from this study are consistent with numerous field and growth chamber experiments showing that P. fluorescens inoculants can increase plant production under ambient CO2,14–15,31, and show that these largely stimulating effects could be additive when combined with eCO2. Although it was impossible to exclude potential fertilization effects on plant available N through bacterial cell addition, the increases in total soil N pool by bacterial cell addition (0.016%) is likely negligible in comparison with the increase in plant growth by an average of 42%. In addition, chemical adjustments to litter C:N may contribute to reduced quality and decomposability of plant litter under eCO2,9,34 which may reduce soil decomposition rates. In comparison to our previous study, overall plant C:N ratios during the rapid vegetative growth stage were lower than during the reproductive stage.3

Plant roots play an essential role in regulating acquisition of soil nutrients. Nevertheless, little is known about the relationship of root functional traits with plant N use strategies. Root surface area is generally correlated to plant nutrient uptake rates.36, P. fluorescens inoculants significantly increased root surface area under eCO2 (Table S1), suggesting that P. fluorescens inoculants could enhance the potential for plant roots to acquire N under N-limited eCO2 condition. Moreover, our results demonstrated that root surface area positively correlated with plant C:N ratio across experimental treatments (Figure 2a), further indicating that the plants’ ability to acquire N from the soil could be influenced by soil N availability.

Coupled C and N processes in the rhizosphere play a critical role in maintaining the sustainability of ecosystems.3,4,57. Recent studies suggest that plant productivity slows when plant N demand decouples from soil C cycling under climate change or other ecological disturbances.38,39. In our previous study conducted in the same ecosystem as this present work, we found that eCO2 increased microbial biomass N immobilization and decreased soil N availability.9,39. However, this study revealed that eCO2 significantly decreased soil enzyme C:N stoichiometry (Table S1), indicating greater microbial demand for soil N under eCO2. N limitation could ultimately dampen ecosystem C sequestration in terms of the eCO2 fertilization effect on plant productivity9,34. However, P. fluorescens inoculant did not directly affect soil enzyme C:N stoichiometry (Table S1). Moreover, the negative relationship between plant biomass C:N and enzyme C:N (Figure 2b) suggests that plants can continue to grow through increase in their capacity to store C per unit of N in response to changes in soil N availability, which is mediated by soil microbial activities.

It is well known that plants can alter their N uptake rates to cope with plant physiological and environmental changes.41–43. By using the 15N isotopic method, we observed that eCO2 increased the importance of N mineralized from SOM, indicating that the positive effects of eCO2 on soil N enzyme activities increased soil N availability (Figure 3a and Table S1). However, P. fluorescens inoculants had no detectable effects on δ15N values of plant biomass (Figure 3a) or on soil N enzyme activities (Table S1). This suggests that P. fluorescens does not facilitate N mineralization under eCO2 conditions.
We note that N in *P. fluorescens* cells is mostly in organic form with the $^{15}$N value of 4.2% (the $^{15}$N value of organic N in SOM is 58.7%). If plants took up mineralized N from dead *P. fluorescens* cells, the $^{15}$N value of plant biomass should be lower than plant uptake. However, if all bacterial N was absorbed by plants, the N in *P. fluorescens* cells would only contribute from 0.8% to 2.2% of the total plant N pool. In addition, the $^{15}$N value of organic N corrected by bacterial cells (58.7%) was still much higher than inorganic N (445.7%).

*eCO2*-induced rhizosphere priming effects and subsequent microbial N mineralization could influence the magnitude of plant growth alterations. Previous studies have identified several plant and microbial traits related to RPE4,21,43, but the direct evidence of priming-related effects on plant N availability has not been well documented. Our results, for the first time, clearly demonstrated that priming of SOM decomposition was positively related to plant N availability (Figure 3b), suggesting that priming made soil N more available to the plant for uptake. Increased root length was also observed in this study as an important root functional trait related to plant N uptake adaptations associated with microbial plant growth-promoting properties under eCO2 conditions. These results add to a growing body of evidence that plants could increase N availability through rhizosphere priming and development of root systems to alleviate nitrogen limitation under eCO24,8,21,43.

*P. fluorescens* inoculants and eCO2 were expected to increase plant C inputs to soil (Figure 4a). However, a synergistic effect of bacteria and eCO2 on plant-derived C was not observed (Figure 4a). This may be due to the use of planting pots which may have constrained root growth in this experiment. Results from a meta-analysis suggest that CO2-induced increases in belowground biomass are stronger in plants grown in open fields relative to closed pots9. In spite of higher root lengths in *P. fluorescens* inoculated treatments across a range of plant species.

### Methods

#### Experimental setup

The C4, perennial grass *Bouteloua gracilis* was selected in this experiment because it is a widespread grass in North America and accounts for most ecosystem net primary productivity in the shortgrass prairie of the central and southern Great Plains46,47. We collected soils for this experiment from the USDA-ARS Central Plains Experimental Range, Colorado, USA. Prior to the experiment, the initial soil inorganic N (NH$_4$+ + NO$_3$-) and organic N (total soil N minus soil inorganic N) content were 0.14 ± 0.02 and 0.41 ± 0.03 mg g$^{-1}$, respectively (n = 8; t-statistic < 0.0001). The $^{15}$N values of inorganic and organic N were 4.57 ± 12.5 and 587.5 ± 10.8%, respectively (n = 8; t-statistic < 0.0001). Inorganic $^{15}$N was determined using the diffusion method45. Organic N was estimated as the difference between total and inorganic N, and organic $^{15}$N was determined by mass balance. The $^{15}$C value of soil organic matter was −20.4% and no inorganic C was present.

We performed our experiment in climate-controlled growth chambers (Percival PGC-9/2, Percival Scientific, Perry, IN, USA). The chamber systems we used were shown to have high reliability and stability during comparative studies of plant genetics and eco-physiology46,47. To simulate field conditions during the growing season, the growth chambers were set to a 14 h daytime period with light intensity of 700 μmol m$^{-2}$ s$^{-1}$. The daytime and night-time temperatures were 25 °C and 18 °C, respectively. We used a Li-250 light meter (LI-COR, Lincoln, NE, USA) and Telaire 7001 meters (Telaire, Goleta, CA, USA) to ascertain the reliability of light intensities and temperatures of the chambers every day. To achieve continuous $^{13}$C-labeling of plant tissues, the chambers were modified to receive an influx of $^{13}$CO$_2$-depleted CO$_2$ (δ$^{13}$C = −33.1%) combined with an external air input which had been scrubbed by a 70-L gas tight soda lime column. The CO$_2$ concentrations inside the chambers were calibrated by infrared CO$_2$ sensors (GMM220, Vaisala, Helsinki, Finland) and continuously monitored by Telaire 7001 meters (ambient CO$_2$ concentration: 371.9 ± 2.1 ppm (mean ± se)); elevated CO$_2$ concentration: 702.9 ± 8.7 ppm). The δ$^{13}$C value of CO$_2$ inside chambers was continuously monitored by a Picarro G2101i-15CO$_2$ analyzer (Picarro, Sunnyvale, CA, USA). Throughout the experiment, the δ$^{13}$C values of CO$_2$ were stable (ambient CO$_2$: −25.0 ± 0.2‰; elevated CO$_2$: −24.9 ± 0.2‰), and there was no significant daily difference in the δ$^{13}$C values of experimental chambers.

We used 24 planted pots with six replicates for each treatment: ambient CO$_2$ without bacteria addition (Control), ambient CO$_2$ and with bacteria addition (B), elevated CO$_2$ and without bacteria addition (eCO$_2$), elevated CO$_2$ and with bacteria addition (B + eCO$_2$). Correspondingly, another 24 unplanted pots were set up with six replicates for each treatment. Three seedlings were transferred to each planted pot.
after the emergence of the first euphylla on moist filter paper in glass Petri dishes. During the first week of the experiment, all pots (including unplanted plots) were after the emergence of the first euphylla on moist filter paper in glass Petri dishes.

We harvested 30 days after planting, because plants have high rates of interactions with soil processes during the rapid vegetative growth stage7,10. We placed each pot (planted and unplanted pots) in an opaque, capped PVC chamber (45-cm height, 20-cm diameter). Briefly, we sealed the bottom of chamber by placing it on a plastic dish containing water to impede gas loss, and removed CO2 inside each chamber by circulating air through a gas tight in-line soda-lime scrubber that, the gravimetric water content in each pot was maintained at 15% (approximately 22 g water). The C in the inoculants if needed) and 

d13Croot biomass = Croot (d13Croot − d13Cstart) / (d13Croot − d13C biomass) 

where Croot is total amount of SOC at the end of the experiment, d13Croot is the d13C value of the root resin, the total soil respiration in the planted treatments and the mean value of soil respiration in the corresponding unplanted treatments, respectively. For each CO2 treatment, the d13C value of the root resin was determined by growing plants in a SOM-free sand.

We calculated the amount of new soil C (Cend) from plant-derived C through rhizodeposition during the experiment using the following model20:

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where Croot is total amount of SOC at the end of the experiment, d13Croot (corrected by the C in the inoculants if needed) and d13Cstart each are the d13C values of SOC at the start and end of the experiment, and d13C biomass is the d13C values of root biomass. A subsample of fresh soil from each pot was used to assess microbial community attributes. To normalize activity to the size of the microbial community, specific microbial respiration was calculated as the ratio of SOC decomposition rate (d13C) to microbial biomass C (MBC). MBC was determined by the fumigation–extraction method and the factor of MBC calculation was 0.451. To stoichiometrically link plant nutrient availability and microbial-mediated SOM decomposition, we measured soil enzyme activities involved in the cycling of C (β-Glucosidase and β-D-Cellulobiosidase) and N (N-acetyl-β-Glucosaminidase and Leucine aminopeptidase) cycling. The enzyme activities were measured using a 4-methylumbelliferyl (MUB) substrate yielding the highly fluorescent cleavage products MUB upon hydrolysis49.

Statistical analyses. To determine the effects of eCO2 and bacteria on plant productivity and soil C/N cycling, we used a two-way ANOVA with eCO2 and bacteria as fixed effects by SPSS 13.0. Post hoc means were determined using least squares means separation by SPSS. The significance level (P value) of post-hoc LSD (least significant difference) test was set to 0.05. Data not meeting assumptions of normality and homogeneity of variance were log-transformed before statistical testing. Simple regression was performed to evaluate relationships underlying rhizosphere processes by Sigma Plot 10.0. Significant effects are reported at P < 0.05 unless otherwise stated.

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**Author contributions**

M.N. and E.P. conceived and designed the experiments. M.N. and C.B. performed the experiments. M.N., C.B. and E.P. analyzed the data. M.N., C.B., M.D.W. and E.P. wrote the manuscript.

**Additional information**

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