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Trade-off between microbial carbon use efficiency and specific nutrient-acquiring extracellular enzyme activities under reduced oxygen

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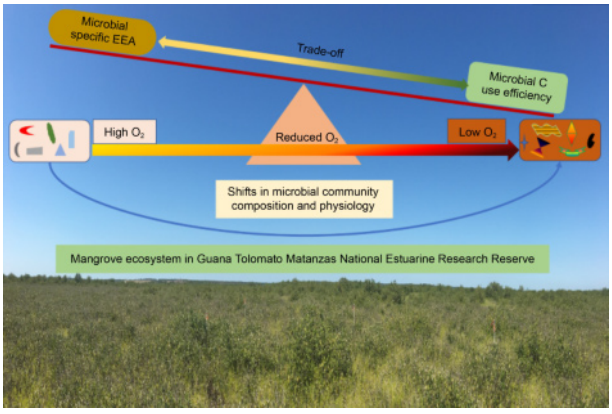
ABSTRACT

- Reduced oxygen increased microbial metabolic quotient (qCO_2).
- Reduced oxygen enhanced microbial specific C-, N- and P-acquiring enzyme activity.
- Reduced oxygen increased microbial C relative to N and P limitation.
- Reduced oxygen increased microbial N relative to P limitation.
- Specific enzyme activity was positively related to qCO_2 under reduced oxygen.



Mangroves are one of the most ecologically sensitive ecosystems to global climate change, which have cascading impacts on soil carbon (C), nitrogen (N) and phosphorus (P) cycling. Moreover, mangroves are experiencing increasing N and P loadings and reduced oxygen availability due to intensified climate change and human activities. However, both direct and interactive effects of these perturbations on microbially mediated soil C, N and P cycling are poorly understood. Here, we simultaneously investigated the effects of N and P loadings and reduced oxygen on microbial biomass, microbial respiration, and extracellular enzyme activities (EEAs) in mangrove soils. We calculated the microbial metabolic quotient (qCO_2), which is regarded as a useful inverse metric of microbial C use efficiency (CUE). Our results show that reduced oxygen significantly increases both qCO_2 and microbial specific EEAs (enzyme activity per unit of microbial biomass) for C-, N- and P-acquisition regardless of N or P loadings. Furthermore, we found that qCO_2 positively correlated with microbial specific EEAs under reduced oxygen, whereas no clear relationship was detected under ambient oxygen. These results suggest that reduced oxygen increases microbial specific EEAs at the expense of increasing microbial respiration per unit biomass, indicating higher energy cost per unit enzyme production.

Keywords reduced oxygen, extracellular enzyme, microbial respiration, nutrient acquisition, nutrient addition, mangrove

1 Introduction

Mangroves have been recognized globally as one of the most carbon (C) rich ecosystems although they only occupy about 0.1% of the Earth's land surface (Bouillon et al., 2008; Donato et al., 2011). Mangroves are regarded as an important C sink due to their waterlogged conditions, high sedimentation rates, high primary productivity, unique root structures, and anoxic soils, which result in low C decomposition rates (Jardine and Siikamäki, 2014; Atwood et al., 2017). For example, it is estimated that mangroves globally store about 5.0−10.4 Pg soil C (Duarte et al., 2013). As such, reducing soil C loss from mangroves potentially represents one of the most cost-effective strategies for mitigating climate change (Siikamäki et al., 2012). However, the patterns and drivers

of soil C cycling in mangroves are not fully understood, which limits our ability to manage mangroves as soil C sinks.

Due to their high primary productivity, mangroves require large amounts of nutrients to support growth, but mangrove ecosystems are characterized by low nutrient availability (Feller et al., 2009; Keuskamp et al., 2013). In fact, nitrogen (N) and phosphorus (P) have been identified as nutrients most likely limiting the primary productivity of mangroves (Lovelock et al., 2006). In recent decades, N and P loadings to mangroves have substantially increased due to intensified human activities and coastal development, but the effects on soil C, N and P cycling are unclear (Feller et al., 2009; Keuskamp et al., 2013). Nitrogen and P loadings have been reported to increase plant primary productivity and associated organic matter inputs to soils (Naidoo, 2009), but effects on organic matter decomposition are uncertain (Keuskamp et al., 2013; Hayes et al., 2017; Simpson et al., 2020). Apart from low nutrient availability, mangrove soils may be varied with oxygen availability, for example, aerobic and anaerobic processes (Behera et al., 2017; Liu et al., 2021), which differ greatly in decomposition rate (Chapman et al., 2019). Also, mangrove areas that are currently exposed at low tide will be underwater for longer periods in progressive tidal cycles due to rising sea level (Cohen et al., 2018), which will have cascading effects on soil C, N and P cycling due to oscillating aerobic and anaerobic conditions (Friesen et al., 2018). Fluctuating soil oxygen availability will make the effects of N and P loadings on soil C, N and P cycling even more complex, making it imperative to investigate the separate and interactive effects of N and P loadings and oxygen availability on soil C, N and P cycling.

Soil microorganisms and extracellular enzymes play essential roles in modulating soil C, N and P cycling (Holguin et al., 2001; Sinsabaugh and Follstad Shah, 2012; Chen et al., 2018), and preferentially invest resources for enzyme production to acquire resources that are limiting growth (Allison et al., 2010; Ren et al., 2018; Wang et al., 2022). For example, soil microorganisms will primarily allocate C and N for phosphatase production when P limits their growth (Jian et al., 2016; Chen et al., 2018; Chen et al., 2020b). However, enzyme production for nutrient acquisition is energetically and C costly, which can couple or decouple microbial C, N and P cycling under different conditions (Mooshammer et al., 2017). The microbial metabolic quotient (qCO_2), the ratio of microbial respiration to microbial biomass, is reported to evaluate microbial C use efficiency (CUE) (Spohn and Chodak, 2015). If soil microorganisms invest more C and energy for nutrient acquisition, this will result in higher qCO_2 and lower microbial CUE. It has been hypothesized that soil microorganisms would likely decrease CUE to maintain metabolic activity when adapting to unfavorable conditions (Moore et al., 2021; Yang et al., 2022; Zhai et al., 2022). However, it remains unclear whether soil microorganisms will shift their CUE to cope with both N and P loadings and reduced oxygen. Meanwhile, external N and P loadings have substantially altered microbial C, N and P cycling by altering nutrient stoichiometry, and are anticipated to have impacts on microbial CUE and enzyme production (Jian et al., 2016; Chen et al., 2018). For example, N loading increased microbial phosphatase production in many ecosystems (Chen et al., 2020b), and was expected to decrease CUE (Widdig et al., 2020). In addition, both microbial CUE and enzyme production are highly sensitive to many biotic and abiotic factors (Spohn and Chodak, 2015; Li et al., 2020; Widdig et al., 2020), such as soil pH, nutrient availability, soil moisture, and microbial biomass. However, the separate and interactive effects of N and P loadings and reduced oxygen on microbial CUE and enzyme production are unclear, impeding predictions of mangroves' ecological functions under changing climate scenarios.

To address the effects of N and P loadings and reduced oxygen on microbially mediated soil C, N and P cycling, we used data from a laboratory incubation experiment that was designed to test the diversity and structure of mangrove soil bacterial communities under these conditions (Craig et al., 2021). Here, we addressed the following research aims that were not examined in the original article; 1), we tested for the effects of reduced oxygen on microbial CUE and microbial specific extracellular enzyme activities (EEAs) across different N and P loading treatments, 2) we explored the relationships between microbial CUE and specific EEAs, and 3) we documented the underlying factors affecting microbial CUE and specific EEAs.

2 Materials and methods

2.1 Study site

The study site is located in the Guana Tolomato Matanzas National Estuarine Research Reserve, St John's County, Florida (29.729° N, 81.242° W). The climate is characterized as humid subtropical, with mean annual temperatures of 16.1°C (min), and 27.2°C (max), and mean annual precipitation of 1317 mm (Dangremond et al., 2020; Simpson et al., 2020). This site is within the saltmarsh-mangrove ecotone, where mangroves have recently expanded into saltmarsh due to a decrease in winter freeze events (Cavanaugh et al., 2014). The region is mainly dominated by low stature (< 1.5 m) and multi-stemmed shrubs of *Avicennia germinans* with a saltmarsh understory dominated by *Batis maritima* and *Sarcocornia perennis*. The site has peaty soils with approximate soil total C, N and P content of 223.0, 10.8 and 0.4 g kg^{-1}, respectively. Detailed information on the study site can be found in Craig et al. (2021).

2.2 Soil sampling and laboratory incubation

Soil samples were collected from an area of about 100 m^2 in April 2018 when the soil surface was exposed at low tide, so that soil samples could contain a mix of aerobic and anaerobic

microbes. Because soil oxygen concentration decreases substantially with depth in mangroves, soil samples were only collected from the top 10 cm of the soil profile. Plant litter, shells and other large debris were manually removed from the soil and the soil was then thoroughly mixed to minimize heterogeneity.

Immediately after soil collection and homogenization, approximately 72 g of soil was added to each of 40 × 473 mL (16 oz.) Ball® smooth sided jars fitted with gas tubing and stop-cock valves in the lid. Jars were randomly assigned to one of two oxygen (ambient and reduced) and one of four nutrient (control, + N, + PK, + NPK) treatments in a fully crossed factorial design with five replicates for each combined treatment. To enable oxygen exchange, the valves were left open in the ambient oxygen treatment even when lids were fitted. The lids for the ambient oxygen treatments were removed for a few minutes and then refitted immediately prior to CO_2 measurement to ensure that ample fresh air was getting into the mesocosms. To mimic anaerobic conditions with the reduced oxygen treatment, jars were flushed with N_2 gas and the valves were then closed. The jars were left to settle for four days prior to administering the nutrient treatments. Ammonium nitrate (NH_4NO_3) was selected as N fertilizer, while potassium phosphate dibasic (KH_2PO_4) as a P and potassium (K) fertilizers. In brief, 2 mL of 4.95 M NH_4NO_3 or 1 mL of 0.16 M KH_2PO_4 were added as N or PK fertilizers, which corresponded to 3.75, 0.07, 0.08 g kg^{-1} N, P and K, respectively. Deionised H_2O was added to each treatment to ensure the same amount of 3 mL liquid inputs for each treatment. These jars were then incubated at 22°C in a dark room for 15 days, which was the constant temperature setting of the laboratory in which the work was conducted. Soil moisture content at the start of the experiment was approximately 83% on a wet weight basis. To minimise moisture fluctuations, water was added mid-experiment.

2.3 Laboratory analysis

Detailed information on the laboratory analysis can be found in Craig et al. (2021). In brief, soil gravimetrical moisture content was recorded based on oven-dried soil samples at 105°C for 48 h. Fresh soil samples were added to deionised water in a 1:2 ratio. Soil pH was measured with an Accumet pH meter (Fisher Scientific, Pittsburgh, PA, USA). Soil total C and N were analyzed following dry combustion method using a Vario EL cube CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Soil total P content was measured following a loss on ignition method by combusting soils at 550°C for 4 h and then extracted using 0.5 M H_2SO_4. Soil P content in the acid extracts was measured colorimetrically on a CLARIOstar microplate reader (BMG LABTECH, Ortenberg, Germany).

To estimate microbial respiration, daily CO_2 flux from each treatment was measured with a LICOR-8100A (Li-Cor Inc., Lincoln, Nebraska, USA). In brief, the first CO_2 measurement from each jar was conducted within half an hour of the N and P or water additions, and each morning thereafter. Four consecutive flux readings of a minute each were taken. To exclude variation at the beginning of CO_2 measurement and to ensure flushing of the gas lines, the last three CO_2 readings were averaged for further analysis, which corresponds to 60-240 s since the closure of the system. At the end of the incubation, microbial biomass C (MBC) was measured based on the chloroform fumigation-extraction method (Brookes et al., 1985) using a TOC-L analyzer (Shimadzu, Kyoto, Japan). An extraction efficiency factor of 0.45 was adopted to estimate MBC (Brookes et al., 1985). Microbial oxidative C-degrading EEAs (peroxidase and phenol oxidase, Table S1) were assayed using L-DOPA method (DeForest, 2009). Microbial hydrolytic C-degrading EEAs (*β*-glucosidase, *β*-xylosidase, cellobiohydrolase) and microbial N- and P-acquiring EEAs (N-acetyl-*β*-glucosaminidase and acid phosphatase, respectively) were analyzed following the method of pNP-linked substrates (Jackson et al., 2013). All soil EEAs measurements were measured colorimetrically using an EZ Read 400 microplate reader (Biochrom Ltd., Cambridge, UK). All soil EEA assays were performed within two weeks after soil sampling. More detailed methods for each enzyme assay can be found in Craig et al. (2021).

2.4 Data analysis

Vector analysis of soil EEAs was adopted to investigate microbial nutrient limitation. Vector length (Eq. (1)) shows microbial C relative to N and P limitation, while the vector angle (Eq. (2)) indicates P relative to N limitation (Moorhead et al., 2016; Liu et al., 2020). Specific C-, N- and P-acquiring enzyme activities were calculated as the ratio of the corresponding EEAs to MBC.

$$\text{Vector length} = \sqrt{(BG/(BG+NAG))^2 + (BG/(BG+AP))^2} \quad (1)$$

$$\text{Vector angle} = \text{Degrees}\left(\text{ATAN2}\left(BG/(BG+AP), BG/(BG+NAG)\right)\right) \quad (2)$$

All data analysis and plotting were performed in R 3.6.2 (R Core Team, 2019). All original data used in this study were published by Craig et al. (2021). All data were first tested for distributional normality using the Shapiro–Wilk method and equality of variances using the Levene test at $p < 0.05$ and transformed when necessary. A linear mixed-effects (LME) model using the "lme" function in the "nlme" package (Pinheiro et al., 2017) was used to evaluate the effects of oxygen and nutrient treatments on the studied variables. Oxygen and nutrient treatments and their interactive effects were considered as fixed effects and each jar as a random effect. The separate effects of oxygen or nutrient treatments within each nutrient or oxygen level on these variables were also evaluated using LME, with oxygen or nutrient treatments as the fixed effects and each jar as a random effect. The Tukey's post hoc test was used to evaluate differences between each paired treatment. Mixed regression analysis

was conducted to explore the relationships between studied variables with each jar as a random effect. The R-squared value of mixed regression models was calculated using the "r.squaredGLMM" function in the "MuMIn" package (Barton and Barton, 2015). To meet statistical requirements, residuals were examined for normality and the residual variances were examined for homogeneity for all models.

3 Results

3.1 Soil carbon, nitrogen and phosphorus content and soil pH

Results of soil C, N and P content and soil pH were published in Craig et al. (2021). In brief, reduced oxygen significantly decreased soil total N content by 8%, while having no effect on soil total C and P content across all nutrient levels (Fig. S1; Table S2). There were significant interactive effects of oxygen and nutrient treatments on soil total N content. Specifically, + N and + NPK under reduced oxygen significantly decreased soil total N content by 17% and 8% as compared to the same treatment under ambient oxygen, respectively. Reduced oxygen increased soil C:N by 6% and decreased soil N:P by 9% across all nutrient levels. In addition, reduced oxygen significantly increased soil pH by 0.87 across all nutrient levels, and the significant differences remained when compared for each nutrient level (Fig. S2).

3.2 Microbial metabolic quotient and specific extracellular enzyme activity

Averaged across the four nutrient levels, reduced oxygen significantly increased qCO_2 by 205% (Fig. 1; Table S3) and microbial specific hydrolytic C-, oxidative C-, N- and P-acquiring EEAs by 122%, 99%, 109%, and 57%, respectively (Fig. 1). Under ambient oxygen, nutrient loadings had no effect on qCO_2 and microbial specific hydrolytic C-, oxidative C-, and N-acquiring EEAs, but showed some small negative impacts on P-acquiring EEA. In contrast, + NPK substantially increased qCO_2, microbial specific hydrolytic C-, oxidative C-, N- and P-acquiring EEAs under reduced oxygen. Furthermore, changes in microbial specific oxidative C- and hydrolytic C-, N- and P-acquiring EEAs were positively related to qCO_2 under reduced oxygen (Fig. 2), but not under ambient oxygen.

Under ambient oxygen, + NPK significantly increased vector length by 3% compared to control, whereas + P alone reduced length compared to both N treatments but not the

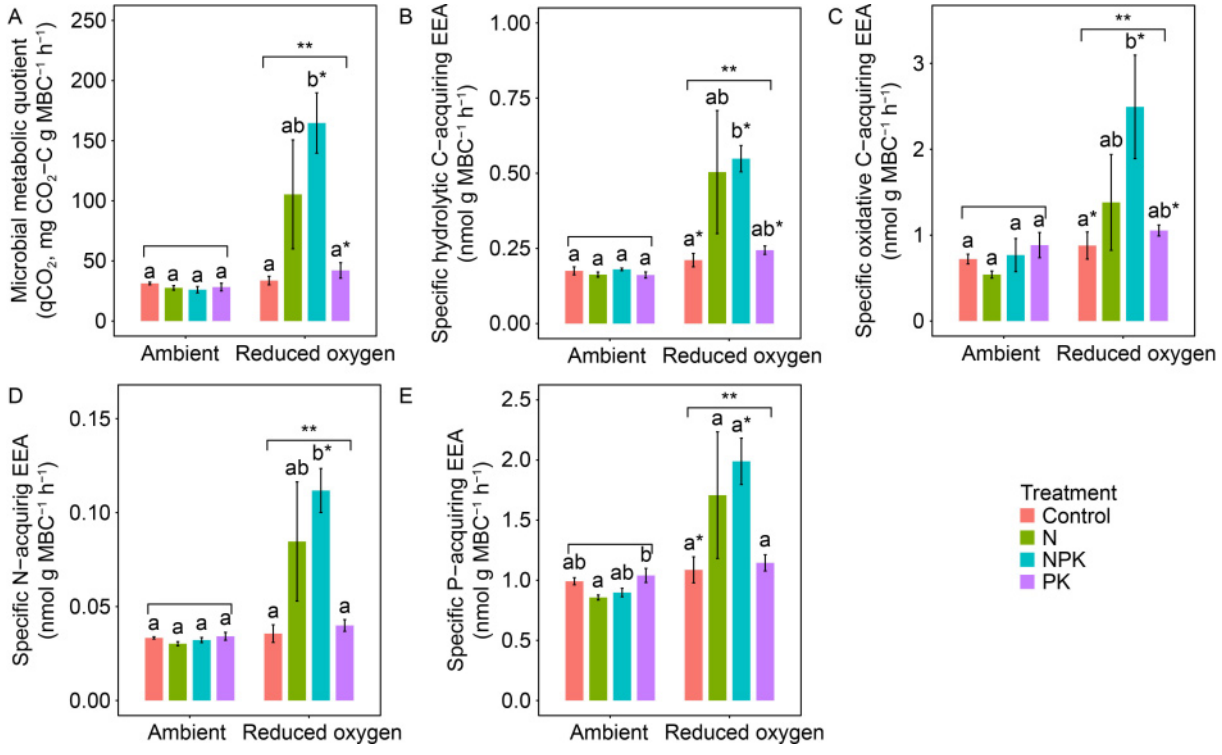


Fig. 1 Effects of nutrient and oxygen treatments on (A) microbial metabolic quotient (qCO_2), (B) specific hydrolytic C-acquiring, (C) specific oxidative C-acquiring, (D) specific N-acquiring, and (E) specific P-acquiring extracellular enzyme activity (EEA). Double asterisks above the braces indicate significant differences (α = 0.05) between ambient and reduced oxygen treatments when different nutrient levels are considered as random factors. Single asterisks above the error bars next to the lowercase letters show significant differences between ambient and reduced oxygen treatments for each nutrient level. Different lower-case letters describe the differences between various nutrient levels for either ambient or reduced oxygen treatments. Values are mean ± standard error for five replicates.

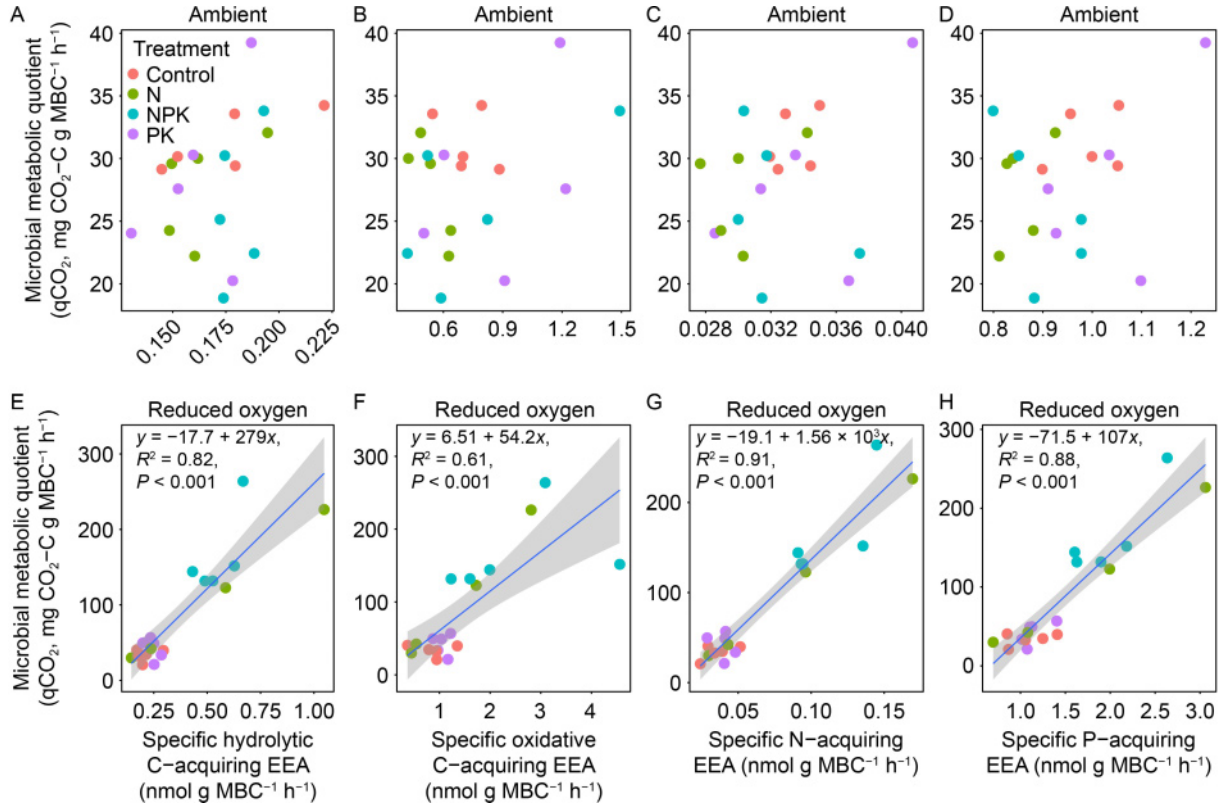


Fig. 2 Relationships between microbial metabolic quotient (qCO_2) and specific extracellular enzyme activity (EEA) for hydrolytic C-, oxidative C-, N-, and P-acquisition under (A−D) ambient and (E-H) reduced oxygen treatments.

control. In contrast, nutrient loading had no effect on vector length under reduced oxygen (Fig. 3; Table S4), although overall vector length was greater than it was under ambient oxygen. Reduced oxygen increased vector length by 4% for control and by 6% for + PK but decreased it by 3% for + NPK when compared to the corresponding nutrient treatments under ambient oxygen. Vector angle generally decreased with + N; + NPK significantly decreased vector angle by 2% compared to control under ambient oxygen, and + N and + NPK respectively decreased this measure by 3% and 4% compared to control under reduced oxygen. Overall vector angle declined under reduced oxygen and when separately compared to the nutrient loading treatment under ambient oxygen, reduced oxygen significantly decreased vector angle by 3%, 3% and 2% for + N, + NPK and + PK, respectively. The enzyme vector angle was negatively correlated with vector length under ambient oxygen, whereas there was no clear relationship under reduced oxygen (Fig. 3). Apart from a negative relationship between enzyme vector angle and qCO_2 under reduced oxygen, there were no clear relationships between vector values and qCO_2 (Fig. 4).

4 Discussion

Our results provide novel evidence of trade-off patterns between microbial CUE and specific EEAs under reduced oxygen in mangroves (Craig et al., 2021). Our results suggest a higher resource cost per unit C-, N-, and P-acquiring enzyme production under reduced oxygen, possibly decreasing microbial CUE and potentially reducing soil C stock over the long-term. The trade-off relationships between microbial CUE and specific soil C-, N-, and P-acquiring EEAs could be used to adjust microbial parameters in models and predictions if a dynamic rather than fixed cost of C investment for nutrient acquisition was explicitly considered. For example, by using soil enzymes as indicators of microbial nutrient requirements and metabolic activities, Wang et al. (2022) developed a dynamic enzyme allocation framework in the Microbial-ENzyme Decomposition model (MEND), which substantially improved modeling projections of soil C and N fluxes in response to N loadings.

4.1 Trade-offs between microbial CUE and specific soil EEAs under reduced oxygen

Our results show that reduced oxygen significantly increases microbial specific C-, N- and P-acquiring EEAs and that qCO_2 was positively correlated with specific EEAs under reduced oxygen. Our results indicate a trade-off between microbial CUE and specific soil EEAs, which is in line with other studies (Ferenci, 2016; Malik et al., 2019; Garcia et al., 2020). For example, soil microbial communities adapted to chronic N deposition can tolerate high levels of N loading, but have lower microbially mediated organic matter

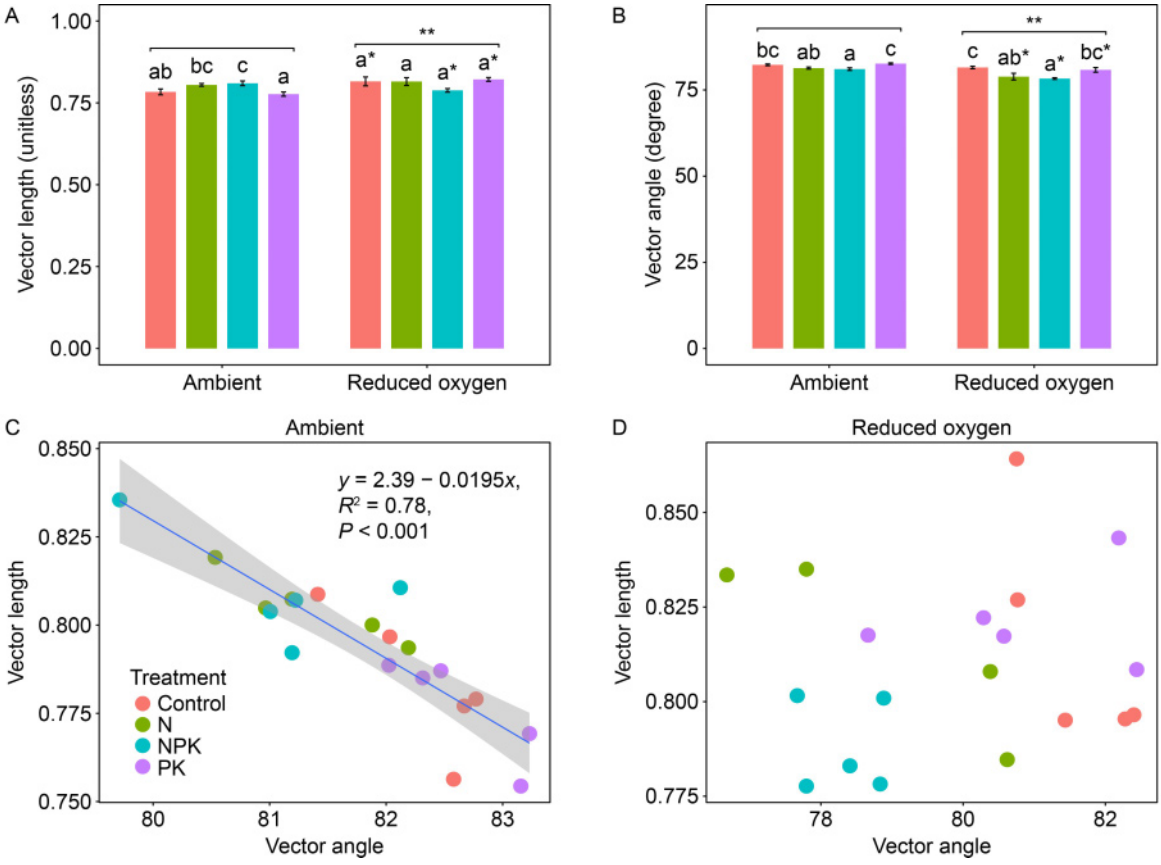


Fig. 3 Effects of nutrient and oxygen treatments on enzyme (A) vector length and (B) vector angle. Relationships between enzyme vector length and vector angle under (C) ambient and (D) reduced oxygen. Double asterisks above the braces indicate significant differences (α = 0.05) between ambient and reduced oxygen treatments when different nutrient levels are considered as random factors. Single asterisks above the error bars next to the lowercase letters show significant differences between ambient and reduced oxygen treatments for each nutrient level. Different lower-case letters describe the differences between various nutrient levels for either ambient or reduced oxygen treatments. Values are mean ± standard error for five replicates.

decomposition and lower microbial CUE (Moore et al., 2021). We next provide three possible explanations for the novel trade-off patterns between microbial CUE and specific soil EEAs under reduced oxygen.

First, there was a sharp drop in MBC with reduced oxygen, indicating that a smaller surviving microbial pool produced the observed levels of microbial metabolic activity compared to a larger microbial pool at ambient oxygen. Additionally, only 37% shared bacterial sequence variants were detected between bacterial communities at ambient and reduced oxygen, as reported by (Craig et al., 2021), and shifts in microbial community composition in response to reduced oxygen have been previously observed (DeAngelis et al., 2010; Pett-Ridge et al., 2013). Perhaps the shifts in microbial community composition could contribute to the different relationships between qCO_2 and microbial specific EEAs with oxygen treatments. Microbes surviving reduced oxygen may gain more stress tolerance at the expense of high resource cost (Moore et al., 2021), which would increase relative microbial respiration, particularly maintenance respiration (Hoehler and Jørgensen 2013; Schimel et al., 2007; Domeignoz-Horta et al., 2020). This explanation is in line with earlier studies showing that reduced oxygen significantly increased energy costs for protein turnover, membrane repair, nutrient ion exchange and movement, leading to a higher qCO_2 (Dijkstra et al., 2011; Han et al., 2011). It should be noted that the relationships between qCO_2 and microbial specific EEAs under reduced oxygen might be related to the sharp decline in MBC, which may result in mathematical rather than biological correlations. But this uncertainty will not weaken our main conclusions because reduced oxygen increased both qCO_2 and microbial specific EEAs. In addition, changes in microbial physiology may also contribute to higher qCO_2 (Brune et al., 2000). For example, reduced oxygen increased the degradation of structurally complex soil C, which was associated with lower microbial CUE, while decreasing litter-derived C decomposition (Huang et al., 2020).

Second, reduced oxygen could exacerbate microbial nutrient limitation through decreased nutrient pool size or reduced microbial nutrient accessibility. For example, in this same study, Craig et al. (2021) reported a significant decline in soil total N content with reduced oxygen when different nutrient levels were considered as random factors. One explanation might be that enhanced enzyme production with reduced oxygen would increase microbial N consumption

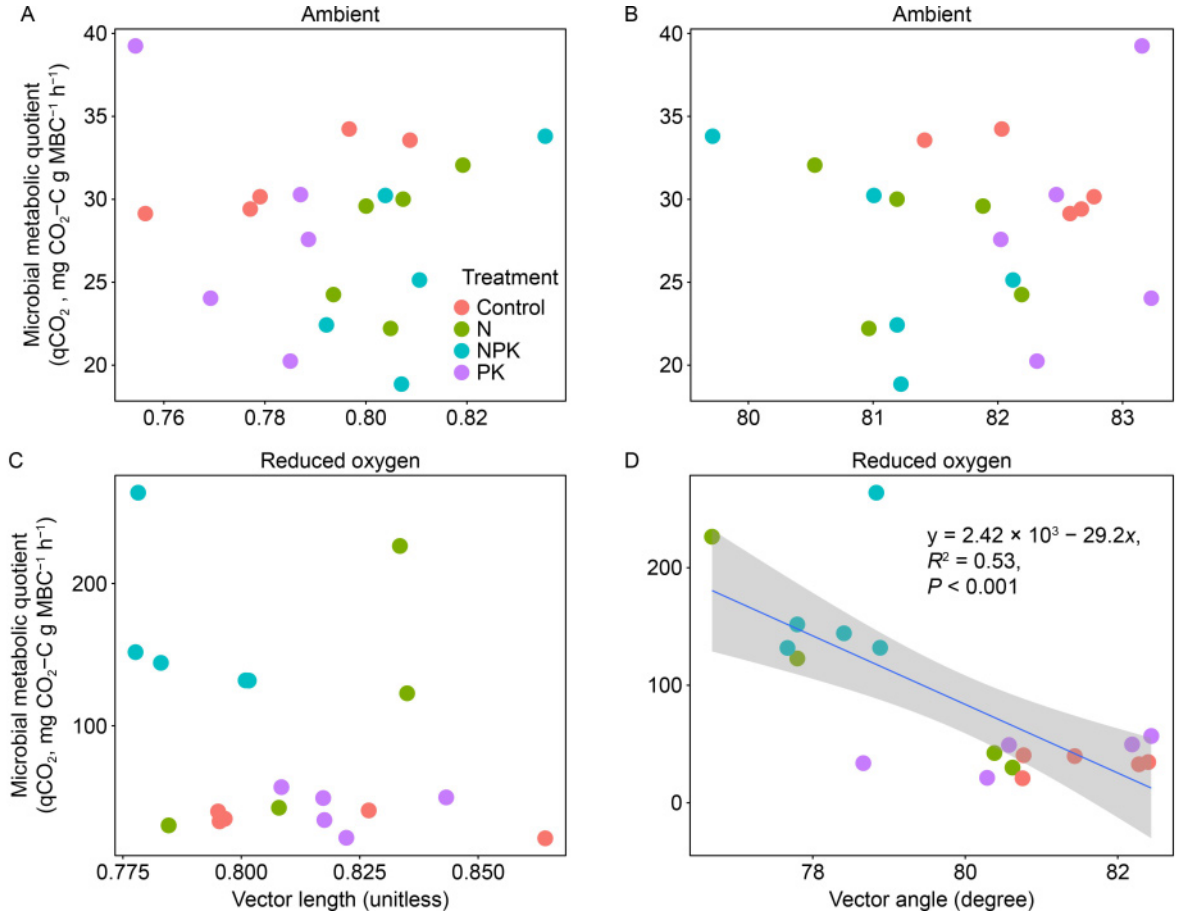


Fig. 4 Relationships between microbial metabolic quotient (qCO_2) and (A, C) enzyme vector length and (B, D) enzyme vector angle under ambient and reduced oxygen.

because enzymes are fundamentally N-rich proteins (Moorhead et al., 2016; Chen et al., 2018). Another explanation might be that reduced oxygen suppressed nitrification but sustained or even increased denitrification and anammox processes (Rysgaard et al., 1994), which may contribute to soil N losses and microbial N limitation. For example, N-loss via anammox increased significantly with the water table level in water saturated and unsaturated riparian zones (Wang et al., 2019). In addition, reduced oxygen favors anaerobes which are reported to be less efficient in nutrient acquisition compared to aerobes (Möller and Müller, 2012). Thus, soil microorganisms will enhance production of N- and P-acquiring enzymes even at a higher resource cost (Schimel et al., 2007; Han et al., 2011). This notion is supported by our findings that: (1) reduced oxygen significantly enhanced specific N- and P-acquiring EEAs even with external N and P loadings (Fig. 1); and (2) shifts in microbial community composition with reduced oxygen were closely correlated with N- and P-acquiring EEAs (Craig et al., 2021).

Third, reduced oxygen significantly increased enzyme vector length, suggesting increased microbial C relative to N and P limitation (Moorhead et al., 2016). This is supported by the co-stimulation of specific hydrolytic and oxidative C-acquiring EEAs with reduced oxygen (Fig. 1), indicating increased decomposition of both labile and recalcitrant C pools (Chen et al., 2020a). Due to the lack of labile C inputs in our laboratory incubation, soil microorganisms must utilize the structurally complex recalcitrant C pools, which are associated with low microbial CUE (Chen et al., 2020a). For example, reduced oxygen can supress the decomposition of litter-derived C but increase mineral-associated C decomposition (Huang et al., 2020), likely through enhanced oxidative C-acquiring EEAs (Freeman et al., 2001). In addition, a large amount of N- and P-containing macromolecules are chemically and physically shielded by lignified macromolecules (Chen et al., 2018; Cui et al., 2020). To meet microbial nutrient demands and balance stoichiometry, soil microorganisms will need to invest more resources to oxidative C-acquiring enzyme production and the associated release of lignin-bound N and P.

4.2 Effects of nutrient loadings on microbial specific EEAs

Nutrient loadings generally had no effect on microbial specific C- and N-acquiring EEAs and qCO_2 under ambient oxygen (Fig. 1), suggesting decoupled responses of MBC, microbial respiration and absolute EEAs (Fig. 2). The study site was not limited by P availability (Dangremond et al., 2020; Craig et al., 2021), despite relatively large P limitation as indicated by overall high vector angles (Fig. 3B).

However, the strong negative relationship between vector length and angle argued for a strong interaction between C (length) and N (angle) acquiring EEAs at ambient oxygen (Fig. 3C), which can also occur if microbes increase utilizing organic N compounds for their C content (Mori, 2020). However, resource limitations (C, N and P) at ambient oxygen, as evidenced by EEAs, appear to be somewhat independent of energy flow, as evidenced by qCO_2. In contrast, under reduced oxygen, nutrient loadings substantially altered microbial specific C-, N- and P-acquiring EEAs and qCO_2, indicating coupled responses of MBC, microbial respiration and absolute EEAs to nutrient loadings (Fig. 2). Moreover, the consistently positive linear relationships between qCO_2 and each specific EEA, without any apparent relationship among relative energy and nutrient acquisition (Fig. 3D), suggests that energy flow (qCO_2) may closely control nutrient demands (EEAs), which in turn are somewhat independent of each other.

These divergent patterns in coupled and decoupled responses at different oxygen levels were emphasized by direct comparisons between qCO_2 and enzyme vectors, i.e., qCO_2 was negatively correlated with vector angle across various nutrient levels under reduced oxygen, whereas there were no relationships otherwise (Fig. 4). Surprisingly, there was no clear relationship between qCO_2 and enzyme vector length under reduced oxygen (Fig. 4C) despite significant relationships between qCO_2 and each independent EEA (Fig. 2). However, the lack of any relationship between vector length and angle under reduced oxygen (Fig. 3D) confirms that relationships between qCO_2 and C-, N-, and P-acquisition vary with oxygen availability. Indeed, both coupled and decoupled responses of MBC, microbial respiration and absolute EEAs to nutrient loadings have been reported elsewhere (Mooshammer et al., 2017; Feng et al., 2019), and depend on how soil microorganisms acclimate or adapt to new environments. Future studies are needed to further explore the microbial functional traits that drive coupled vs. decoupled microbially mediated resource acquisition and nutrient cycling and how these traits will respond to nutrient loadings and reduced oxygen.

We found strong interactive effects of nutrient loadings and reduced oxygen on microbial specific C-, N-, and P-acquiring EEAs and qCO_2 under reduced oxygen (Table S3). No previous studies have simultaneously investigated the effects of nutrient loadings and oxygen levels on these variables in mangrove ecosystems. However, our results were in line with studies from other environments showing that soil microorganisms were more sensitive to nutrient loadings under environmentally unfavorable conditions than under ambient conditions (Ferenci, 2016; Garcia et al., 2020). Indeed, microbially mediated resource acquisition and nutrient cycling depend on the resistance and resilience of soil microorganisms to changing environmental conditions and nutrient loadings (Ng et al., 2015; Mooshammer et al., 2017). Given the strong interactive effects of reduced oxygen and nutrient loadings on MBC, microbial respiration and soil EEAs, accurate predictions of the responses of biogeochemical cycles to these factors in a changing world require the explicit consideration of specific environmental conditions.

4.3 Uncertainties and implications

There are several limitations and uncertainties in our study. First, only a few related studies have concurrently investigated MBC, microbial respiration and EEAs under reduced oxygen in mangroves, limiting the comparison of our results to other studies from mangroves. It is possible that our results may differ with studies from other different ecosystems. For example, N loading significantly increased soil pH and decreased soil phosphatase activity due to the unique soil redox conditions in the studied mangrove ecosystem (Craig et al., 2021), which contrasts with studies from many other ecosystems (Jian et al., 2016; Chen et al., 2020b). These inconsistent results highlight the value of our study for advancing the understanding of an understudied ecosystem. Second, the laboratory incubation used in the present study did not fully represent in situ microbial respiration due to soil disturbance, short-term incubation, and lack of plant-derived C inputs. Thus, future research may consider incubating intact soil cores for a longer duration. Third, there are several different kinds of C-, N-, and P-acquiring enzymes, whereas only BG, NAG and AP were considered in enzyme vector analysis. One reason for this selection was to follow the classical enzyme studies (Sinsabaugh et al., 2008) and vector analysis (Moorhead et al., 2016), so that our results are comparable. Moreover, different kinds of enzymes with shared ecological functions or within the same group usually respond similarly to experimental treatments, which may reduce the uncertainties when calculating the enzyme vectors (Chen et al., 2017). Fourth, microbial CUE is the ratio of C allocated to growth and C taken up by microorganisms (Spohn and Chodak, 2015), but it varies with scale and methods of calculation, such as our use of qCO_2, so that caution is required when comparing studies (Geyer et al., 2016; Schimel et al., 2022). In addition, shifts in soil microbial community composition are thought to explain the trade-off between microbial CUE and specific EEAs, but direct evidence linking changes in specific microbial communities and to microbial respiration is lacking. To further explore the links between microbial community composition, CUE and EEAs, integration of state-of-the-art microbial functional gene abundance and advanced statistical analysis are needed (Chen and Sinsabaugh, 2021).

Despite the abovementioned uncertainties, our study provides an indication that soil microorganisms may change their community composition, physiology, and nutrient requirements to adapt to reduced oxygen, leading to trade-offs between microbial CUE and specific EEAs. However, this trade-off is not explicitly considered in either contemporary experimental or modeling frameworks, generating substantial uncertainties in predicting soil C cycling.

Moreover, this trade-off has implications for relationships between microbially mediated soil C and nutrient cycling, with potential to advance the parameterization of biogeochemical cycling. Future research is required to explicitly explore the underlying microbial, edaphic, and environmental mechanisms associated with this trade-off between microbial CUE and specific EEAs.

5 Conclusion

Our results advance on our previous work (Craig et al., 2021) by demonstrating the trade-off between microbial CUE and specific EEAs under reduced oxygen, suggesting a higher energy cost per unit enzyme production. This relationship can substantially advance the understanding of microbially mediated C and nutrient cycling. For example, Allison et al. (2010) significantly improved model projections of soil C dynamics by considering the relationship between microbial CUE and enzyme production. However, this trade-off has not been resolved in experimental or model frameworks to predict soil resource acquisition and nutrient cycling in anaerobic ecosystems. In addition, shifts in microbial community composition may play essential roles in microbial enzyme production under reduced oxygen, underscoring the need for more advanced research on microbial community composition. Given the large areas of global anaerobic ecosystems and their huge amount of C stocks, more research on the relationships between microbial CUE and specific EEA and the underlying mechanisms are needed.

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Electronic supplementary material

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