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RESEARCH ARTICLE

Nutrient-induced changes in root respiration in 10 woody plant species

Deping Zhai[1,2] | Ana Clarissa Negrini[2,3,4] | Joana Zaragoza-Castells[5] | Kristine Y. Crous[6] | Odhran S. O'Sullivan[7] | Patrick Meir[8] | Kevin L. Griffin[9] | Matthew H. Turnbull[10] | Xuhui Zhou[1,11] | Owen K. Atkin[2,3]

[1]Center for Global Change and Ecological Forecasting, Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China; [2]Division of Plant Sciences, Research School of Biology, The Australian National University, Canberra, Australian Capital Territory, Australia; [3]ARC Centre of Excellence in Plant Energy Biology, Research School of Biology, The Australian National University, Canberra, Australian Capital Territory, Australia; [4]Department of Agriculture, Water and the Environment, Australian Government, Canberra, Australian Capital Territory, Australia; [5]Department of Geography, Faculty of Environment, Science and Economy, University of Exeter, Exeter, UK; [6]School of Science and Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia; [7]Department of Animal and Plant Sciences, The University of Sheffield, Sheffield, UK; [8]School of Geosciences, University of Edinburgh, Edinburgh, UK; [9]Department of Earth and Environment Sciences, Columbia University, Palisades, New York, USA; [10]School of Biological Sciences, University of Canterbury, Christchurch, New Zealand and [11]Northeast Asia Ecosystem Carbon Sink Research Center (NACC), Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, School of Forestry, Northeast Forestry University, Harbin, China

Correspondence
Owen K. Atkin
Email: owen.atkin@anu.edu.au

Matthew H. Turnbull
Email: matthew.turnbull@canterbury.ac.nz

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Abstract

1. Nitrogen (N) and phosphorus (P) are soil macronutrients that influence ecosystem productivity through strong impacts on plant metabolism. The influence of nutrient supply on the relationships between leaf respiration rate (*R*) and leaf N concentration ([N]) has been widely investigated. By contrast, how root *R* responds to variations in nutrient availability and whether there remains a general response across a wide range of species is less well known.
2. We conducted an experiment assessing the effects of N and P supply on root *R* in 10 woody plant species, with root *R* being determined by the in vivo rate of oxygen (O_2) consumption. Maximum *R* (R_{max}) was also quantified by O_2 uptake in the presence of an exogenous substrate and a respiratory uncoupler
3. Our results showed that high-N and high-P supply significantly stimulated mass-based root *R* in woody plants, with the effects of N supply significant only when P supply was high. The promoting effect of high-P treatment remained consistent despite N supply. Root *R*-[N] bivariate relationships were altered by nutrient availability across all species, with higher root *R* at a given root [N] under low- than high-N supply. Similarly, root *R* at a given P concentration ([P]) was higher under low- than high-P supply. Root R_{max} was significantly higher than in vivo *R* for all nutrient treatments, showing that in vivo root *R* was limited by substrate supply and/or adenylates, with no significant difference in R/R_{max} ratios among nutrient treatments.

4. These results indicate that ecosystem models should consider different scaling relationships linking root *R* to root N or P concentrations for woody species when predicting the effects of nutrient availability on carbon cycle dynamics and climate–biosphere feedback.

KEYWORDS
nitrogen, nutrient availability, phosphorus, respiration capacity, root respiration

1 | INTRODUCTION

Root respiration (*R*), which accounts for 30%–60% of total soil carbon (C) efflux (Chen et al., 2021; Hirano et al., 2023; Jian et al., 2022), is a fundamental process of C exchange. Root *R* can mediate the terrestrial ecosystem C balance via its responses to changes in the environment (Govind & Kumari, 2014). Nutrient availability in soil is known to directly affect root *R* through regulating the demand for respiratory energy (e.g., for nutrient uptake) and indirectly through effects on processes in remote organs, such as the stem or leaves (Eissenstat et al., 2000; Freschet et al., 2021). Nitrogen (N) and phosphorus (P) are two key elements for plant growth (Wang, Cresswell, et al., 2021), with multiple roles in metabolism (Bloomfield et al., 2014; Luo et al., 2013; Vitousek et al., 2010), so it is not surprising that variation in plant N and P concentrations is often linked to changes in respiration rates, both in leaves and roots (Dusenge et al., 2019; Rubio-Asensio & Bloom, 2017).

N-rich roots are likely to exhibit greater *R* because respiratory energy is needed for the acquisition and assimilation of N (Amthor, 2000; Rubio-Asensio & Bloom, 2017) and the increased energy requirements of cellular maintenance in organs/tissues with high protein concentrations (Atkinson et al., 2007; Burton et al., 2012; Eissenstat et al., 2000). However, the extent to which variation in root N is linked to changes in root *R* likely depends on how N is partitioned between metabolically active and inactive components. Past work has shown that N partitioning is crucial in leaves (Evans, 1989), with preferential investment in N-rich proteins maintaining demand for respiratory energy even in tissues with low N concentrations ([N]). Given the functional links between tissue N and respiratory metabolism, strong relationships linking specific *R* and tissue [N] have been reported, both in leaves and roots (Atkin et al., 2015; Atkinson et al., 2007; Burton et al., 2012; Crous et al., 2017; Han & Zhu, 2021; Reich et al., 2008). How relationships between *R* and tissue [N] are influenced by variations in N supply remains less studied, especially for roots.

Root *R* can also be strongly affected by P availability, as low tissue P concentrations ([P]) can increase the level of adenylate restriction of mitochondrial electron transport (Covey-Crump et al., 2002; Gonzalez-Meler et al., 2001; Jarvi & Burton, 2018) and limit the activity of ATP-dependent phosphofructokinase, a key enzyme in glycolysis (Bligny & Gout, 2017; Theodorou & Plaxton, 1993). P limitations can also affect leaf photosynthetic metabolism, resulting in suppressed substrate supply to roots (Hartley et al., 2006; Lewis et al., 1994). Together, the concentration of N and P available in soils and the resultant concentration in tissues should exert a profound influence on root *R*. There is growing evidence that N-mediated changes in *R* also depend on P availability and how P is distributed in cells (Bloomfield et al., 2014; Crous et al., 2017; Lambers & Oliveira, 2019; Meir et al., 2001, 2007). For example, inorganic phosphate (Pi) stored in vacuoles can be transported into the cytosol and subsequently to other organelles when soil P is limiting (Liu et al., 2015; Luan et al., 2017; Wang, Chen, & Wu, 2021), with the result that the cytoplasmic P pools can remain relatively homeostatic in response to changes in P supply (Yang et al., 2017). Crucially, it is the metabolic fraction of total P in the cytoplasm (i.e. the size of physiologically active P pools) that exerts the greatest influence on plant *R*, with the size of the metabolic P pools differing among plant taxa; as a result, the effects of P limitation on *R* of leaves and roots are likely to differ among PFTs (Veneklaas et al., 2012).

Given the functional links between tissue N and respiratory metabolism, strong relationships linking specific rates of respiration and tissue [N] have been reported, both in leaves and roots (Atkin et al., 2015; Atkinson et al., 2007; Burton et al., 2012; Crous et al., 2017; Reich et al., 2008). Leaf R-[N] scaling relationships often shift in response to changes in growth temperature and nutrient availability (Atkin et al., 2015). Burton et al. (2012) showed that root R-[N] correlations altered significantly under contrasting N supply in hardwood forests. In addition, N-deficient plants can allocate a greater fraction of leaf N investment to defence rather than metabolic activities compared with their high-N grown counterparts (Bryant et al., 1983; Crous et al., 2017; Evans & Poorter, 2001; Onoda et al., 2004). Whether the same is true for roots is, however, not known. Moreover, while we know that *R*-[N] scaling relationships differ between leaves and roots (Reich et al., 2008), less is known about the impacts of different nutrient supply on *R*-nutrient scaling in roots compared with that of leaves (Lambers et al., 2002).

Given the additional metabolic roles that N and P play in leaves (e.g. photosynthesis) compared with roots, it is unclear whether the effects of contrasting nutrient supply on root *R* will be similar to those on leaf *R*. In this study, we investigated whether the response of root *R* to nutrient availability (N or P) varies with the other nutrient (P or N) supply in woody plants. Our first hypothesis was that root *R* would be higher in plants grown in high-N or high-P supply compared with low-nutrient supply, with the promoting effect of high-N or high-P supply being more effective when the other nutrient (P or N) is not limiting (Hyp. 1). Second, because respiration

can be significantly impacted by the concentration of key metabolic enzymes which are associated with the level of tissue [N] (Reich et al., 2008), we hypothesized that the effect of nutrient supply on root *R*-[N] (or [P]) relationships will be similar to those reported for leaves (Crous et al., 2017), with low-nutrient availability resulting in an increase in the proportionality coefficient (i.e. intercept of log root *R*-root nutrient relationships), but not the scaling exponent (i.e. slope of log root *R*-root nutrient relationships) (Hyp. 2).

We also assessed whether contrasting N and P supply affected the regulatory control (adenylate/substrate) of root and leaf *R* in woody species, with our study focusing on the effect of nutrient supply on maximum *R* (R_{max}) and the proportion of *R* engaged in vivo (i.e. R/R_{max}). Here, we tested a third hypothesis that R_{max} would be lower in plants grown on low-N or low-P supply and that R/R_{max} would be lower in low-P grown plants (due to greater adenylate restriction of respiration when P is limiting), but not in low-N grown plants (Hyp. 3). Our experiments used a subset of 10 woody species from the same sand/hydroponic-grown plants in a previously published study that reported on leaf-level CO_2 exchange (Crous et al., 2017).

2 | MATERIALS AND METHODS

2.1 | Experimental design

For this study, 10 woody species were used (Table S1) for growth treatments, see Crous et al. (2017). Briefly, seedlings were transplanted into 3.18 L plastic cylinders (50 cm in height and 9 cm in diameter) containing sterilized sand in November 2008 and were measured in June and July 2009. All the seedlings were less than 1 year old when they were transplanted. Plants were divided into four treatment groups, with each group receiving modified Hoagland No. 1 solution (Hoagland & Arnon, 1950) containing specific concentrations of N and P. Here, our aim was to achieve changes in foliar chemistry that were also reflected in rates of leaf metabolism, rather than necessarily provide field-relevant levels of nutrient availability for any of the species in question. N and P were provided at nominally 'high' and 'low' levels in four treatment combinations consisting of high N and high P (HNHP), high N and low P (HNLP), low N and high P (LNHP), and low N and low P (LNLP). The high N solution contained 5 mM KNO_3 and the low N solution 0.4 mM KNO_3 (modified after Atkinson et al., 2007). The high P solutions contained 1 mM KH_2PO_4 (Edwards et al., 2006) whereas low P had 2.0 μM KH_2PO_4 to limit storage of P in the vacuole as a buffer (after Campbell & Sage, 2006). Thus, N:P supply ratios varied from 5:1 for HNHP to 2500:1 for HNLP, 0.4:1 for LNHP and finally 200:1 for LNLP. In addition to N and P, the modified Hoagland solution also contained 0.07 mM Ca_2Cl and 0.45 mM $MgSO_4$, as well as several micronutrients (4.2 μM boron, 1.2 μM manganese, 0.8 μM zinc, 0.03 μM copper, 0.04 μM molybdenum and 0.01 μM cobalt). Each nutrient solution was balanced for cations and iron (Fe) was added as ferric EDTA to a level of approximately 8 μM Fe. These micronutrient concentrations were one-tenth of those in the recommended Hoagland solution because full strength can result in toxic symptoms (Leggett, 1971). Immediately after transplanting, between 120 and 150 mL of nutrient solution was applied to individual plants each day at the rate of 20 mL min^{-1}, pumped from 200-L storage containers which were refilled regularly. All plants and nutrient treatments were randomly assigned to six replicate blocks spread across two adjacent glasshouses (with three replicate blocks in each glasshouse). The glasshouses employed natural light and the growth temperature was maintained at 25°C/18°C day/night.

The experimental approach above was previously shown to be effective in generating a range of leaf-nutrient and functional phenotypes between 'deficient' and 'adequate-abundant' (Crous et al., 2017) and tissue N and P concentrations were consistent with the range of previously published values (Johri et al., 2015; Li et al., 2022). There was no evidence from leaf physiological responses (Crous et al., 2017) of toxicity effects in the HNHP treatments. Additionally, leaf P concentrations (Crous et al., 2017; Table S1) in the two most likely P-sensitive *Hakea* species did not reach levels previously reported as critical for toxicity effects (Shane et al., 2004), with the exception of *Hakea multilineata* in the HNHP treatment only. Roots from this species in this treatment were not included in the analysis.

2.2 | Measurement protocols

Respiration (*R*) measurements were made after 7 months of growth following the commencement of nutrient treatments. Oxygen (O_2) uptake by detached roots was measured polarographically in cuvettes containing 30–40 mL of aerated modified Hoagland's nutrient solution (pH 5.8) buffered with 10 mM MES (2-(N-Morpholino) ethanesulfonic acid) using Clark-type O_2 electrodes (Dual Digital Model 20; Rank Brothers, Cambridge, UK) coupled to a computer-based data acquisition system (NI-DAQ for Windows 2000, National Instruments, Berkshire, UK). The cuvette was maintained at a constant measurement temperature of 25°C in a water bath. Measurements were made in solutions containing the same N and P concentrations in which each plant was grown. Whole roots were removed from the growth cylinders and carefully washed in water. Uniformly metabolically active young roots were sub-sampled for the *R* measurements, as these are responsible for active exploration and nutrient uptake (Liang et al., 2023). Following a 10 min stabilization, O_2 depletion was recorded over a 10 min period, with all measurements terminating before the O_2 concentration was 40% of air saturation.

Following measurements, roots were oven-dried (2 days at 70°C) and the dry mass was recorded. Root [N] and [P] were analysed on dried and ground root samples after Kjeldahl digestion (Sáez-Plaza et al., 2013) using a flow injection analyser (Lachat Instruments, Loveland, CO, USA) for N and P using the indophenol blue and ammonium molybdate methods, respectively. It should be noted that for those woody species that formed proteoid root systems (cluster roots), only non-proteoid roots were selected to evaluate the *R* rate.

Leaf O_2 uptake was measured using a Hansatech Oxygraph (Norfolk, England) O_2 electrode system, using 2 mL cuvettes. Intact leaf discs (cut with a sharpened 0.7 cm^2 leaf corer) from the lamina region of the most recently fully expanded leaves were sliced into 2 mm thick slides while immersed in a measurement buffer [10 mM Hepes, 10 mM MES and 0.2 mM $CaCl_2.2H_2O$ (pH 7.2)]; leaf slices in buffer solution were kept in darkness for 30 min to overcome post-illumination transients and wounding effects (Lantz et al., 2019). Following the measurements of respiratory O_2 uptake (10 min stabilization, followed by O_2 depletion being recorded for several minutes, with measurements terminating before O_2 concentration was 40% of air saturation), samples were oven-dried at 70°C for at least 2 days to determine dry mass.

To assess the impact of nutrient treatments on the proportional engagement of respiratory capacity (R/R_{max}), we measured the rates of O_2 uptake by roots and leaves of woody plants in the combined presence of an uncoupler and exogenous substrate. These were done at the same time as the above measurements using a matched sample from the same plants. We adopted a similar approach to that adopted previously (Atkin et al., 2009; Jiang et al., 2023), with exogenous substrate (50 mM glucose from a 2 M stock) and uncoupler (3 μM carbonyl cyanide m-chlorophenyl hydrazone) prepared as previously described (Covey-Crump et al., 2002). The proportional engagement of respiratory capacity was indicated by the ratio of root R to R_{max} (R/R_{max}).

2.2.1 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Plant	Plot	6 of each nutrient combination

2.3 | Data analysis

All data were log-transformed before analysis due to the non-normal distribution of the raw data. All calculations and analyses were performed using the R language (R Development Core Team, 2015). Initial analysis found no significant effect of finer-scale plant functional types (PFTs) on root R with respect to the impacts of the four nutrient treatments (Figure S1; Table S2); hence, we proceeded with the single 'woody' grouping. Linear mixed effects models (LMMs) were employed to test the fixed effects of nutrient treatments on root respiration and root chemistry, with species included as a random effect to account for interspecific variation (R package: "lme4"; Table 1). Significant effects were tested using the "anova" function in R, implemented through the "lmerTest" package. One-way ANOVA based on LMMs was also used to test the main and random effects on leaf respiration, root and leaf R_{max} rate, and R/R_{max}. Pairwise comparisons were performed using the "emmeans" package in R, based on least-squares means, where significant fixed effects were detected ($p < 0.05$).

Standardized Major Axis (SMA) regression was used to describe bivariate relationships linking root R with root chemistry (Table 2) using log-transformed values. Differences in coefficients of SMA regressions between treatments were also tested using the R package 'smatr' (Warton et al., 2006). SMA models were fitted by treatment groups; if regressions were significant, we then tested whether the slope of the regression for each treatment group was significantly different from each other. If the treatment groups shared a common slope, the significant differences in the intercepts were then tested using the common slope. Post-hoc pairwise comparison was conducted following the test of coefficient significance where there was a significant difference.

3 | RESULTS

3.1 | Effect of nutrient supply on root nutrient content and R

Nutrient treatments had significant effects on the overall means of root [N], root [P], root [N]/[P] ratio and root R across all woody species ($p < 0.05$; Figure 1; Table 1). Additionally, significant random effects of species were observed for root [N] and root R ($p < 0.05$), but not for root [P] and [N]/[P] ($p > 0.05$; Table 1). Specifically, the high-N treatment significantly increased root [N] regardless of P supply, resulting in a 52% increase ($p < 0.05$; Figure 1a). P supply also had a significant effect on root [N], with high-P treatment enhancing root [N] in plants receiving high-N supply, whereas no significant effect of P treatment was observed in plants grown under low-N supply (Figure 1a). High-P supply led to high root [P], but N treatment did not induce significant changes in root [P] (Figure 1b). P treatment also significantly affected root [N]/[P], with values being significantly higher under low- than high-P treatment ($p < 0.05$; Figure 1c). High-N treatment significantly increased root [N]/[P] in plants grown under low-P supply, but no effect was found in plants under high-P supply (Figure 1c).

Root R was significantly impacted by both N and P treatments, but the response of root R to N treatment differed between high- and low-P treatment groups (Figure 1d). High-N supply increased root R by 40% under high-P supply ($p < 0.05$), while it had no significant effect on root R under low-P supply ($p > 0.05$; Figure 1d). High-P treatment significantly increased root R by 67% irrespective of N supply ($p < 0.05$; Figure 1d). Nutrient treatments also significantly impacted leaf R (Table S3), with high-P supply leading to high leaf R, but only in the high-N treatment groups (Figure 1e). By contrast, N treatment had no significant effect on O_2-based leaf R (Figure 1e).

3.2 | Impacts of nutrient supply on relationships between root R and nutrients

To determine whether the relationships linking mass-based root R with root [N] and [P] were affected by nutrient availability, we

constructed log–log plots for root *R* against [N] and [P] (Figure 2). The root *R*-nutrient relationships across woody species varied significantly among the treatment groups, as evidenced by differences in slopes (scaling exponents; Figure 2a; Table 2). The root *R*-[N] relationship exhibited a significantly greater slope in high-N treatment groups than in low-N groups, despite the non-significant regression in the high-N and low-P group (HNLP; Figure 2a; Table 2). *R*-[N] regression slopes did not differ significantly between high- and low-P treatments for plants grown under low-N supply (Figure 2a; Table 2). Significant root *R*-[P] relationships were observed for plants grown under low-N supply, with a greater slope in the low-P treatment group compared with the high-P treatment group (LNHP: 0.45, LNLP: 1.09; Figure 2b; Table 2). There was no significant root *R*-[P] relationship in the two high-N treatment groups (HNHP and HNLP).

3.3 | Proportional engagement of maximum respiratory capacity

As was the case with in vivo respiration, rates of root R_{max} (i.e. respiration measured in the presence of uncoupler and substrate) differed significantly between plants grown on high vs. low N and P treatments, with the effect of N depending on the availability of P and vice versa (Figure 3a; Table 3; for pairwise comparisons between *R* and R_{max}, see Table 4; Figure S3). Leaf R_{max} was affected by P availability but not N (Figure 3b; Table 3). Interestingly, nutrient treatment had no significant effect (Table 3) on root R/R_{max}, which averaged ~0.9 (Figure 3c) or leaf R/R_{max}, which averaged ~0.8 (Figure 3d). Thus, the proportional effect of nutrient supply on *R* largely mirrored that of the effect on R_{max} in leaves and roots.

4 | DISCUSSION

The primary aim of this study was to investigate how nutrient supply affects root *R* and the relationships between root *R* and root nutrients in selected woody plant species. We found nutrient supply-driven changes in root chemistry, root *R* and root *R*-root nutrient relationships. Root [N] and [P] were significantly increased by high levels of nutrient supply. Both high-N and high-P supply significantly stimulated mass-based root *R* in the woody plants (Hyp. 1), with the effect of N treatment being significant only when P supply was high. Responses to nutrient supply were generally similar among a finer categorisation of woody plant functional types (i.e. broadleaved trees, broadleaved shrubs and needle leaved trees; Figure S1). The scaling exponent (slope) of root *R*-root nutrient relationships differed among treatment groups (Hyp. 2), which led to a situation in which root *R* at a given root nutrient concentration was greater in plants receiving low- than high-nutrient supply. High-P supply also significantly increased R_{max} in roots and leaves of woody species, with N treatment only significantly impacting

TABLE 1 One-way ANOVA for differences in root nutrient concentrations (N concentration, [N]; P concentration, [P] and the ratio of [N]/[P]) and root respiration (*R*) among nutrient treatments using linear mixed models with species as a random factor.

Variables	Source of variance		*p*
[N]	Fixed effects	Treatment	<0.01
	Random effects	Species	<0.01
[P]	Fixed effects	Treatment	<0.01
	Random effects	Species	0.07
[N]/[P]	Fixed effects	Treatment	<0.01
	Random effects	Species	0.08
Root *R*	Fixed effects	Treatment	<0.01
	Random effects	Species	<0.01

TABLE 2 Coefficients of Standard Major Axis (SMA) regression for relationships linking root respiration (*R*) with root N concentration ([N]) and P concentration ([P]) shown in Figure 2.

Model	Treatment	R^2	*p*	Slope	Elevation	H_0 for slope	Pairwise com	Figure
Root *R*-[N]	HNHP	0.13	0.03	2.07 (1.50, 2.85)	−1.52 (−2.49, −0.56)	<0.01	A	Figure 2a
	HNLP	<0.01	0.82					
	LNHP	0.21	<0.01	0.86 (0.64, 1.16)	0.30 (−0.01, 0.60)		B	
	LNLP	0.26	<0.01	1.03 (0.78, 1.37)	0.00 (−0.33, 0.34)		B	
Root *R*-[P]	HNHP	0.04	0.23			<0.01		Figure 2b
	HNLP	<0.01	0.58					
	LNHP	0.10	<0.05	0.45 (0.33, 0.62)	0.95 (0.83, 1.08)		B	
	LNLP	0.19	<0.01	1.09 (0.81, 1.45)	1.09 (1.03, 1.16)		A	

Note: Models of root *R*-[N] and root *R*-[P] were fitted by treatment groups across all woody species using the Standard Major Axis (SMA) method, the determination coefficients (R^2) and significance values (*p*-values) are shown for each treatment group. Scaling exponents (slope) and proportional coefficients (elevation) with 95% confidence intervals are also shown if the regressions are significant. Significant differences of the regressions were tested between treatment groups. Scaling exponents significantly differed between nutrient treatments, and post-hoc pairwise differences are indicated via different capital letters. Treatment abbreviations: HNHP (high N high P), HNLP (high N low P), LNHP (low N high P), LNLP (low N low P).

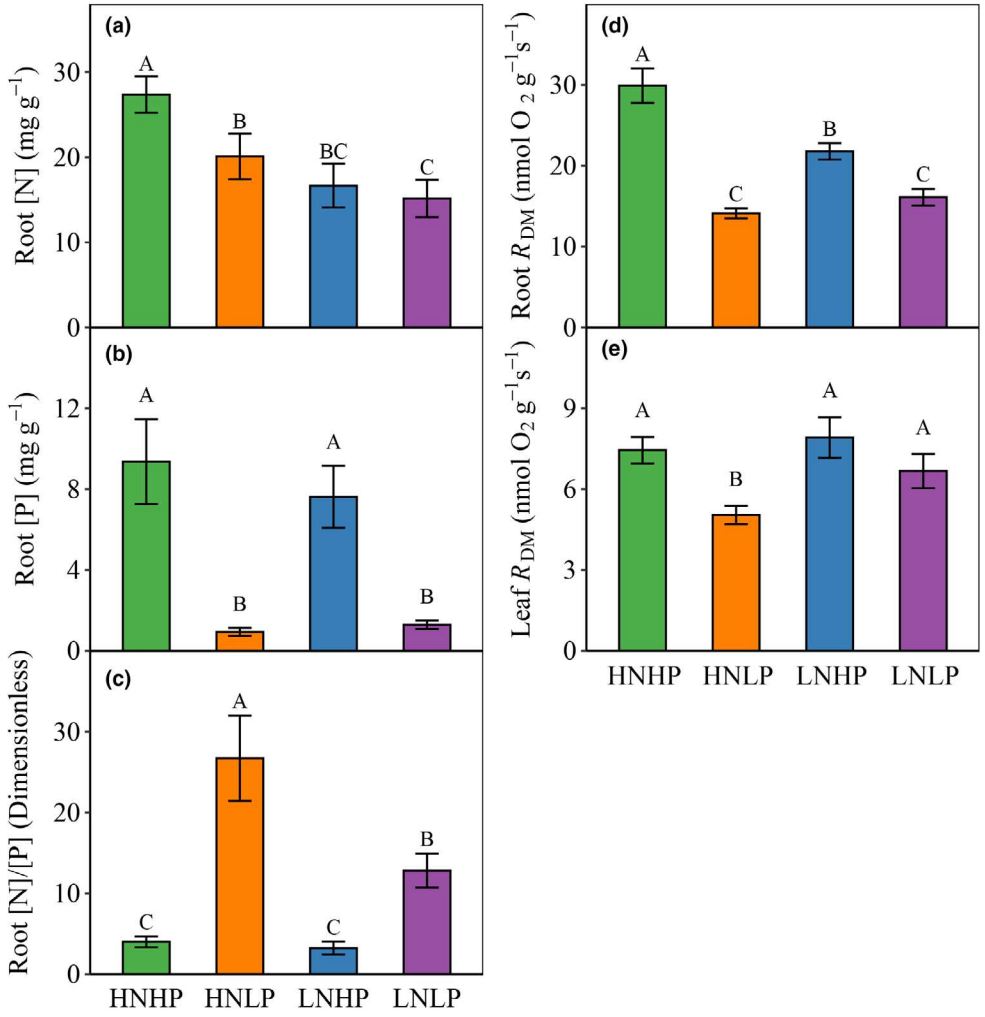

FIGURE 1 Means and standard errors of root nitrogen concentration ([N], (a), root phosphorus concentration ([P], (b), the ratio of root nitrogen to phosphorus concentration ([N]/[P], (c), dry mass-based root respiration (root R_{DM}, (d), and dry mass-based leaf respiration (leaf R_{DM}, (e) of woody species under four nutrient treatments (HNHP: high N high P; HNLP: high N low P; LNHP: low N high P; LNLP: low N low P). Different uppercase letters above the bars show significant differences among the four treatments.

root R_{max} (Hyp. 3). Regardless, nutrient treatment had no impact on the relative in vivo engagement of maximum respiratory capacity (R/R_{max}) (Hyp. 3). Taken together, these results provide strong insights into drivers of root *R*, with important implications as discussed below.

4.1 | Effects of nutrient treatments on root *R*

Supporting Hyp. 1 that root *R* will be higher in plants grown in high-N or high-P supply compared with low-nutrient supply, we found that high-N and high-P supply significantly stimulated root *R* of woody plants, with the effect of N supply depending on the availability of P (Figure 1d). Conceptually, this is supported by previous findings that when N is in limited supply, the demands for respiratory products (ATP, NADPH and C skeletons) to assimilate N are relatively lower, which suppresses root *R* (Nunes-Nesi et al., 2010; O'Leary et al., 2019). Previous reports found increases in N availability significantly increased root *R* (Hasselquist et al., 2012; Van Der Werf et al., 1993, 1994; Zeng et al., 2018). The reason why N supply had no effect on root *R* when P was in low supply could be ascribed to the fact that P deficiency exerts a downregulation of ATP turnover on root *R*, which potentially obscures the effect of N availability (Carstensen et al., 2018; Yang et al., 2021). The inhibitory effect of P deficiency was still evident when N was limiting, but the extent to which low-P supply decreased root *R* was less under low-N supply than high-N supply (Figure 1d). The strong effect of low-P supply on root *R* in woody plants exposed to high-N solution agrees with previously published results for leaves that the response to P addition was strongest when N was abundant (Crous et al., 2017; Meir et al., 2001).

4.2 | Changes in root *R*-root nutrient relationships in response to N and P

Based on Crous et al. (2017), who reported nutrient-mediated changes in leaf *R*-[N] relationships when *R* and [N] were expressed

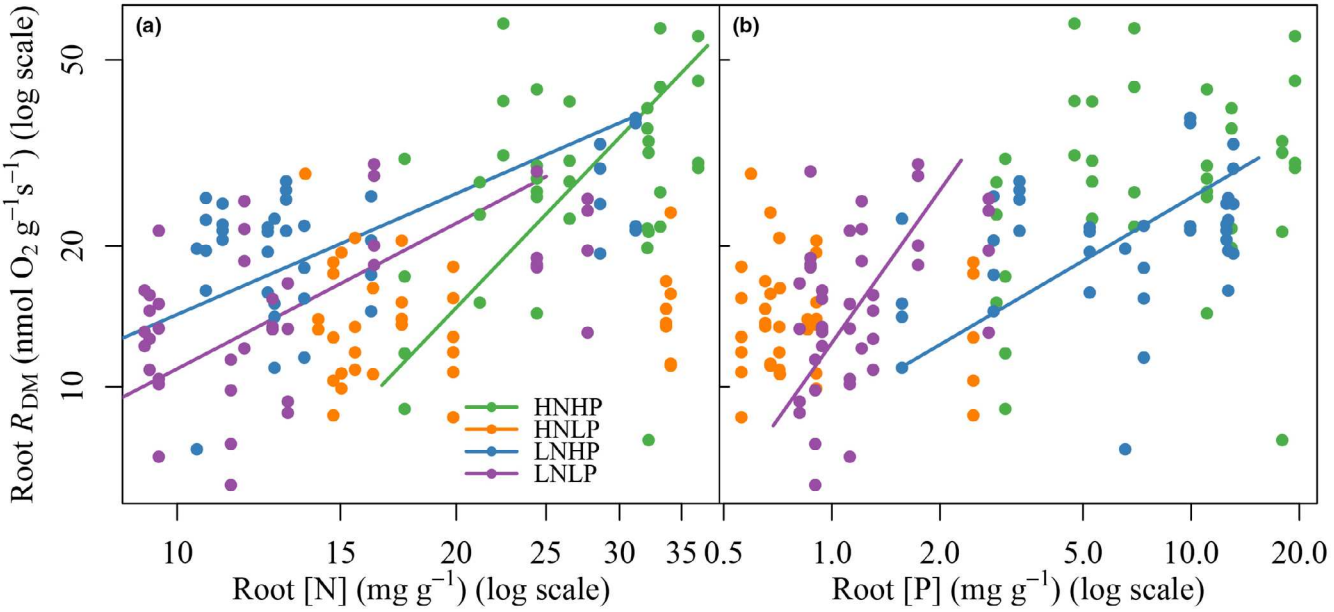

FIGURE 2 Relationships of mass-based root respiration (root R_{DM}) as a function of nitrogen concentration (root *R*-[N], (a) and as a function of phosphorus concentration (root *R*-[P], (b) across woody species. Standardized Major Axis (SMA) regressions were fitted by treatment groups, with regression coefficients reported in Table 2. Different colours indicate nutrient treatments (HNHP: high N high P; HNLP: high N low P; LNHP: low N high P; LNLP: low N low P).

on a leaf area basis, we predicted (Hyp. 2) that low-nutrient supply would increase the proportional coefficient (intercept) but not alter the scaling exponent (slope) of root *R*-[N] relationships (Hyp. 2). Contrary to this hypothesis, we found that nutrient supply significantly altered the scaling exponent of mass-based root *R*-[N] relationships, with low N supply decreasing the slope of the *R*-[N] relationships, irrespective of P supply (Figure 2a; Table 2). One consequence of this change in scaling exponent was that rates of root *R* at a given root [N] were higher in plants grown under limiting N supply, particularly in species that exhibit inherently low [N] values (Figure 2a). Interestingly, in plots of mass-based values of leaf *R* vs. [N], Crous et al. (2017) reported a similar finding—that being that low N supply significantly reduced the scaling exponent of mass-based leaf *R*-[N] relationships, irrespective of P supply. Thus, the phenomenon of low N treatment decreasing the scaling exponent of mass-based *R*-[N] relationships appears to be similar in roots and leaves.

Why would the scaling exponent change under different nutrient treatments? Although little is known of the N reallocation in roots, previous work on leaves (Crous et al., 2017) suggests that low-N treatment may result in the reallocation of N to metabolism, such that a decrease in leaf [N] is not proportionally matched by the decreasing rate of photosynthesis or respiration. Similarly, it is possible that the change in root *R*-nutrient relationships under different nutrient supply might be largely due to the trade-off in organic N reallocation in roots between metabolic and non-metabolic pools (Millard & Grelet, 2010). The change in the root *R*-[N] relationship under low-N supply suggests that the reallocation of N may help maintain metabolic rates when N is deficient (Mantelin & Touraine, 2004).

In addition, high-P supply resulted in significantly higher root *R* at a given [N], but only under conditions of high-N supply (Figure 2a; Figure S1a). A range of factors might contribute to this observation, including the role of P supply in determining the level of adenylate restriction on root *R* (Gonzalez-Meler et al., 2001; Rychter et al., 1992), as well as how P supply affects uptake and assimilation of nitrate by roots, and thus the extent to which N accumulates in roots. As an example of how P supply influences N metabolism, Rufty et al. (1990) found that P deficiency reduced nitrate uptake by roots, which in turn subsequently limited the synthesis of shoot protein in P-stressed plants. Similarly, Gniazdowska and Rychter (2000) found that nitrate uptake and nitrate assimilation in *Phaseolus vulgaris* were both suppressed under low P conditions, with decreases in the latter being greater than the former; as a result, nitrate accumulated to a greater extent in roots under low compared to high P supply. These studies reflect the importance of P for the process of protein synthesis, as well as the effect of P limitations on mitochondrial ATP synthesis, with ATP needed for nitrate transport and amino acid synthesis. Such observations highlight the linkage between N and P metabolism that result in root *R* being greater in high-P grown plants than their low-P counterparts, at least for plants grown under high-N supply. They also provide a possible explanation for why root *R* at a given root [N] is lower under P-limited conditions.

Although there was no significant root *R*-[P] relationship in high-N grown woody plants (Figure 2b), low-P supply significantly decreased the root [P] in all plants (Figure 1b), while root *R* at a given [P] was higher in low-P grown plants than in their high-P grown counterparts (Figure S2b). As a result, rates of root *R* at a given root [P] were higher in P-limited plants. Previous studies have observed

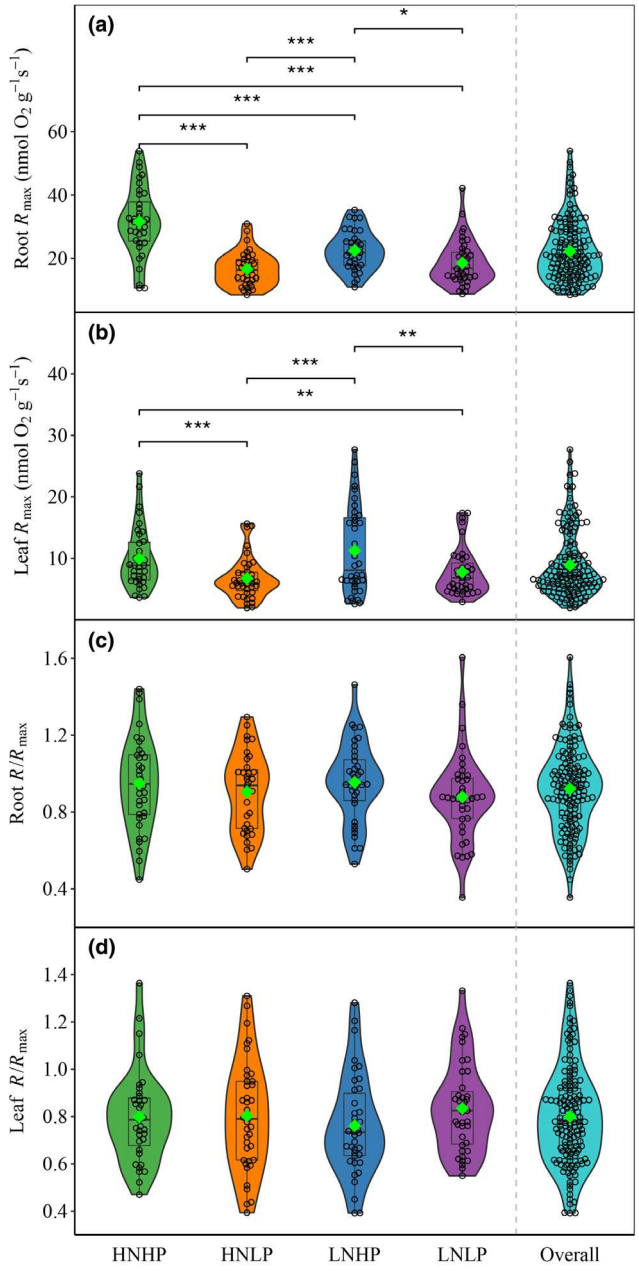

FIGURE 3 Violin plots of maximum respiration (R_{max}, a, b) and proportional engagement of R_{max} (R/R_{max}, c, d) in woody species for roots (a, c) and leaves (b, d) under four nutrient treatments (HNHP: high N high P; HNLP: high N low P; LNHP: low N high P; LNLP: low N low P) and at the whole woody-group level (Overall). The green diamond in each box indicates the mean value for each group. Statistical comparisons between treatments are denoted by horizontal lines with asterisks (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

TABLE 3 One-way ANOVA for differences in maximum respiration (R_{max}) and proportional engagement of R_{max} (R/R_{max}) in roots and leaves of woody species among nutrient treatments, using linear mixed models with species as a random factor.

Items	Source of variance		*p*
Root R_{max}	Fixed effects	Treatment	<0.01
	Random effects	Species	<0.01
Leaf R_{max}	Fixed effects	Treatment	<0.01
	Random effects	Species	<0.01
Root R/R_{max}	Fixed effects	Treatment	0.37
	Random effects	Species	0.93
Leaf R/R_{max}	Fixed effects	Treatment	0.19
	Random effects	Species	<0.01

that when P is limiting, stored inorganic phosphorus (Pi) in vacuoles is translocated into the cytoplasm to compensate for the shortage of metabolically active P, which in turn enables the (relative) maintenance of respiratory metabolism (Liu et al., 2015; Yang et al., 2017). Thus, the higher rates of root *R* per unit P may result from the reallocation of vacuolar P to the cytosol, which in turn enables respiration to continue (albeit at a reduced rate compared to high-P grown plants; Tsujii et al., 2024).

4.3 | The effects of nutrient availability on in vivo engagement of respiratory capacity

The third hypothesis of our study had two components: (a) R_{max} would be lower in plants grown on low-N or low-P supply; and (b) R/R_{max} would be lower in low-P grown plants but not in low-N grown plants. Related to both was the question of whether observed, in vivo rates of *R* were lower than R_{max}; we found that R_{max} was significantly higher than in vivo *R* in both roots (+10%) and leaves (+33%) of woody species (Figure 3; Figure S3; Table 3). At moderate temperatures, the rate of *R* is usually not constrained by the capacity of respiratory enzymes (Atkin et al., 2000; Atkin & Tjoelker, 2003; Covey-Crump et al., 2002). In our study, all O_2 consumption rates were measured at 25°C, which allows achievement of capacity for respiratory enzymes disregarding tissue [N] levels. Thus, our results of the significantly higher R_{max} indicate that in vivo woody root *R* was limited by adenylates and/or substrates (Covey-Crump et al., 2002; Jarvi & Burton, 2018). Importantly, the extent to which in vivo *R* was lower than R_{max} was unaffected by nutrient treatment, with nutrient supply-induced changes in in vivo *R* being matched by concomitant changes in R_{max} (Figure 3; Table 3). Thus, while nutrient supply did indeed influence R_{max} (supporting Hyp. 3(a)), the results do not support Hyp. 3(b).

Low-P supply can lead to adenylate restriction of electron transport in the mitochondrial inner membrane and restricted rates of ATP synthesis (Carstensen et al., 2018; Igamberdiev & Kleczkowski, 2015; O'Leary et al., 2017), potentially reducing in vivo *R*. However, the fact that R/R_{max} was unaffected by nutrient treatment suggests that respiratory capacity scaled with in vivo demand for respiratory products. Past work has shown that plants receiving low-P supply tend to exhibit a relatively slow growth rate (Constan-Aguilar et al., 2014; Varkitzi et al., 2010) and decreased rates of photosynthesis (Chaudhary et al., 2008)—conditions that

TABLE 4 Paired t-test for differences between the in vivo respiration (R) and respiratory capacity (R_{max}) of root and leaf in woody species.

Organ	Treatment group	R	Means ± SE	t	df	p
Root	HNHP	Root in vivo R	29.91 ± 2.13	1.91	35	0.06
		Root R_{max}	31.60 ± 1.78			
	HNLP	Root in vivo R	14.52 ± 0.73	3.15	34	<0.01
		Root R_{max}	16.66 ± 0.92			
	LNHP	Root in vivo R	21.16 ± 1.08	1.86	35	0.07
		Root R_{max}	22.46 ± 1.02			
	LNLP	Root in vivo R	15.93 ± 0.96	3.87	39	<0.01
		Root R_{max}	18.59 ± 1.11			
	Overall	Root in vivo R	20.30 ± 0.82	5.43	146	<0.01
		Root R_{max}	22.26 ± 0.78			
Leaf	HNHP	Leaf in vivo R	7.47 ± 0.51	6.31	34	<0.01
		Leaf R_{max}	9.97 ± 0.84			
	HNLP	Leaf in vivo R	5.03 ± 0.34	5.44	38	<0.01
		Leaf R_{max}	6.75 ± 0.54			
	LNHP	Leaf in vivo R	7.79 ± 0.71	6.84	37	<0.01
		Leaf R_{max}	11.26 ± 1.17			
	LNLP	Leaf in vivo R	6.45 ± 0.60	5.83	38	<0.01
		Leaf R_{max}	7.77 ± 0.63			
	Overall	Leaf in vivo R	6.66 ± 0.29	12.07	150	<0.01
		Leaf R_{max}	8.89 ± 0.43			

Note: The abbreviations for treatments were as follows: High N high P, HNHP; high N low P, HNLP; low N high P, LNHP; and low N low P, LNLP.

would reduce demand for energy under low-P supply, leading to lower investment in cytochrome oxidase, followed by reduced amounts of mitochondrial protein and R_{max}. Hence, one possibility is that the selected plants grown under low P matched ATP capacity with metabolic needs in low-P supply to achieve optimal resource use.

5 | CONCLUSIONS

Collectively, our results suggest mass-based root R was significantly affected both by N and P treatments. The scaling relationships linking root R to root nutrient concentrations shifted when plants received different levels of nutrients, likely underpinned by changes in nutrient allocation within plant tissues. Although low-nutrient supply induced significant inhibitory effects on root R, our study highlights the relatively high efficiency of nutrient usage for root R when nutrients are in limited supply, illustrated by the greater root nutrient-based R under low-nutrient supply. Considering the lack of studies on root R under controlled environments, our findings provide important insights, particularly with respect to the effect of nutrient supply on root R and root R-[N]-[P] scaling relationships. They are of particular relevance to Earth System Models seeking to predict the impact of future climates on below-ground respiration rates with the consideration of nutrient cycles both in soils and plants (Bonan et al., 2002). For such models, further work characterising the impact of nutrient supply across a broader spectrum of species growing in a range of contrasting biomes, along with consideration of the role of ontogeny in regulating root respiration responses to nutrient supply, is needed to test the generality of the relationships observed in our study.

AUTHOR CONTRIBUTIONS

Owen Atkin, Patrick Meir, Matthew Turnbull and Kevin Griffin planned and designed the research. Ana Clarissa Negrini, Joana Zaragoza-Castells, Kristine Crous and Odhran O'Sullivan conducted the experiments. Deping Zhai, Kristine Crous, Xuhui Zhou and Owen Atkin analysed data. Deping Zhai, Owen Atkin, Kristine Crous, Xuhui Zhou and Matthew Turnbull led the writing with substantial contributions from Odhran O'Sullivan, Joana Zaragoza-Castells, Ana Clarissa Negrini and Patrick Meir.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data is available on the Australian National University Research Data portal: https://doi.org/10.25911/r8ja-sz61 (Zhai et al., 2025).

STATEMENT ON INCLUSION

This study is based on experimental work on a range of plants carried out at ANU Canberra with authors from a number of countries. Because this work is not field- or place-based, there are no other 'stakeholders' within the region where the study was conducted.

ORCID

Ana Clarissa Negrini https://orcid.org/0000-0001-5149-9225
Kristine Y. Crous https://orcid.org/0000-0001-9478-7593
Odhran S. O'Sullivan https://orcid.org/0000-0002-1448-1066
Patrick Meir https://orcid.org/0000-0002-2362-0398
Kevin L. Griffin https://orcid.org/0000-0003-4124-3757
Matthew H. Turnbull https://orcid.org/0000-0002-3433-5859
Owen K. Atkin https://orcid.org/0000-0003-1041-5202

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SUPPORTING INFORMATION

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

Table S1: Species means of O_2-based respiration rate and root chemistry.

Table S2: Two-way ANOVA testing for differences between treatment and woody plant functional type.

Table S3: One-way ANOVA testing for differences in O_2-based leaf respiration rates of woody plants among four nutrient treatments.

Figure S1: Means and standard errors of dry mass-based root respiration (Root R_{DM}) (a) and leaf respiration (Leaf R_{DM}) (b) of each plant functional type.

Figure S2: Levels of nitrogen-based and phosphorus-based root respiration in woody species under four nutrient treatments.

Figure S3: Paired plot of the in vivo respiration (R) rate and the respiratory capacity (R_{max}) of leaves and roots in each woody species.

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