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RESEARCH IN CONTEXT

Nitrogen concentration and physical properties are key drivers of woody tissue respiration

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• **Background and Aims** Despite the critical role of woody tissues in determining net carbon exchange of terrestrial ecosystems, relatively little is known regarding the drivers of sapwood and bark respiration.
• **Methods** Using one of the most comprehensive wood respiration datasets to date (82 species from Australian rainforest, savanna and temperate forest), we quantified relationships between tissue respiration rates (R_d) measured *in vitro* (i.e. 'respiration potential') and physical properties of bark and sapwood, and nitrogen concentration (N_{mass}) of leaves, sapwood and bark.
• **Key Results** Across all sites, tissue density and thickness explained similar, and in some cases more, variation in bark and sapwood R_d than did N_{mass}. Higher density bark and sapwood tissues had lower R_d for a given N_{mass} than lower density tissues. R_d–N_{mass} slopes were less steep in thicker compared with thinner-barked species and less steep in sapwood than in bark. Including the interactive effects of N_{mass}, density and thickness significantly increased the explanatory power for bark and sapwood respiration in branches. Among these models, N_{mass} contributed more to explanatory power in trunks than in branches, and in sapwood than in bark. Our findings were largely consistent across sites, which varied in their climate, soils and dominant vegetation type, suggesting generality in the observed trait relationships. Compared with a global compilation of leaf, stem and root data, Australian species showed generally lower R_d and N_{mass}, and less steep R_d–N_{mass} relationships.
• **Conclusions** To the best of our knowledge, this is the first study to report control of respiration–nitrogen relationships by physical properties of tissues, and one of few to report respiration–nitrogen relationships in bark and sapwood. Together, our findings indicate a potential path towards improving current estimates of autotrophic respiration by integrating variation across distinct plant tissues.

Key words: Autotrophic respiration, CO_2 efflux, metabolic nitrogen, physical properties, sapwood respiration, stem respiration, structural nitrogen, tissue density, tissue thickness, woody tissue respiration

INTRODUCTION

Autotrophic respiration is the dominant contributor to terrestrial ecosystem respiration (68 ± 3 %; Campioli *et al.*, 2016) and, as such, it is likely to be an important driver of variation in net carbon exchange of these ecosystems under a changing climate (Duffy *et al.*, 2021). In a global analysis of forest species, respiration from leaves, above-ground woody tissues (sapwood and bark) and roots of saplings and mature trees were estimated to contribute 39 ± 4, 22 ± 3 and 38 ± 4 % to autotrophic respiration, respectively (Campioli *et al.*, 2016). In this study we investigated drivers of respiration for two of those three categories: leaves and above-ground woody tissues.

Understanding the main drivers of autotrophic respiration is crucial to modelling ecosystem carbon fluxes. Leaf 'dark' respiration (R_d) in terrestrial biosphere models is typically represented as a function of temperature and either leaf tissue nitrogen (N) concentration (N_{mass}) or photosynthetic capacity (Atkin *et al.*, 2017). A positive relationship between leaf N and R_d has been demonstrated extensively, within (Ryan, 1995; Reich *et al.*, 1996; Vose and Ryan, 2002) and among species (Reich *et al.*, 1998; Wright *et al.*, 2006; Atkin *et al.*, 2015). The strong dependence on N_{mass} is indicative of the major contribution of protein turnover to leaf R_d [*ca.* 10–60 % but as high as 90 % (Penning de Vries, 1975; De Visser *et al.*, 1992; Amthor, 2000; Cannell and Thornley, 2000)] but is also indicative of

other metabolic processes that scale with N_{mass}, such as synthesis and phloem loading of photosynthates. Other main contributors to leaf R_d are the energetic costs associated with maintaining ionic gradients between cellular compartments, turnover of lipid membranes, protecting the photosynthetic apparatus against damage from high light and repairing damage when this does occur (Amthor, 2000; Cannell and Thornley, 2000; Millar *et al.*, 2003).

The drivers of woody tissue respiration are considerably less understood but, again, N (amino acids and soluble proteins) is thought to play a central role, as do various metabolic processes [e.g. conversion and storage of non-structural carbohydrates (NSCs)] occurring in the living cells of sapwood and inner bark (Penning de Vries, 1975; Ryan, 1991; Reich *et al.*, 2008). The inner bark, i.e. the secondary phloem, consists of multiple metabolically active layers including the parenchymatous cortex and the phellogen (or cork cambium) which, in turn, gives rise to the outer bark. The inner bark is associated with the transport and storage of photosynthates and secondary compounds, while the outer bark, which includes only dead cells, offers mechanical stability, reduced water loss and protection from fire, physical injury and pathogens (Paine *et al.*, 2010; Rosell, 2016, 2019). Compared with sapwood, the paucity of knowledge regarding bark physiology and ecology is surprising given that – at least in trees – the total bark surface area per unit ground area may be as high as *ca*. 30–50 % of the total leaf surface area (Whittaker and Woodwell, 1967), which could have important consequences for stand-level gas exchange.

From relevant studies of woody tissue respiration (Sprugel, 1990; Pruyn *et al.*, 2002*a*, *b*, 2003; Cavaleri *et al.*, 2006; Spicer and Holbrook, 2007*a*; Katayama *et al.*, 2014), the following key generalities emerge: respiration (per mass or volume) of inner bark is up to an order of magnitude higher than that of sapwood; respiration tends to decrease from the outer layers inwards; heartwood respiration is low but not zero; and sapwood respiration rates are generally higher in branches than in main trunks. There are various causal factors that explain these spatial patterns. First, concentrations of N and NSCs are generally higher in inner bark than in sapwood, and are higher in the outer sapwood than in the inner sapwood (Pruyn *et al.*, 2005; Rodríguez-Calcerrada *et al.*, 2015). Non-structural N compounds, i.e. amino acids and soluble proteins, also accumulate more in outer sapwood relative to inner sapwood (El Zein *et al.*, 2011). Ray parenchyma cells, which transport NSCs and nutrients in and out of storage and have high respiratory activity relative to other cell types, are more active in outer than in inner sapwood (Gartner *et al.*, 2000). Lastly, inner sapwood is probably more oxygen limited than outer sapwood. Oxygen to the sapwood is supplied by the transpiration stream (xylem sap flow) and can vary with stem position (Eklund, 2000), and – compared with the inner sapwood – the outer sapwood is in closer proximity to the metabolically active vascular cambium (which separates bark from sapwood) and to the bark itself: O_2 diffuses inwards through lenticels in the bark and may also be generated via bark cortical photosynthesis (Cernusak *et al.*, 2006).

Commonly, woody tissue respiration is measured as the efflux of CO_2 (or sometimes O_2 influx) using chambers attached directly to the surfaces of tree trunks (boles), i.e. over the bark (Ryan *et al.*, 1995, 1996; Cernusak *et al.*, 2006). CO_2 efflux measured this way is comprised of several fluxes (Teskey *et al.*, 2017): sapwood respiration, bark respiration, bark photosynthesis [e.g. in twigs but also in boles, for some smooth-barked species (Cernusak and Hutley, 2011; Rosell *et al.*, 2015)] and, importantly, flux from CO_2 dissolved in the xylem sap. This last flux can be positive (CO_2 arriving from the roots; Bloemen *et al.*, 2013) or negative (CO_2 transported to leaves), and quite sizeable. For this reason, stem CO_2 efflux is often measured at night or at dawn, when xylem flow is minimal. Partitioning the observed CO_2 efflux among various woody tissues (sapwood and bark) and processes is certainly possible, but challenging (McCree, 1986; Ryan *et al.*, 1995, 1996; Stockfors and Linder, 1998; Cernusak *et al.*, 2006; Pérez-Priego *et al.*, 2014). An alternative approach (used in this study) is to measure CO_2 efflux on sapwood or bark material excised from trees using an increment borer or hammer and chisel (Pruyn *et al.*, 2002*a*, *b*, 2003, 2005; Spicer and Holbrook, 2005, 2007*a*, *b*). In that literature, the measured quantity is sometimes referred to as 'respiration potential', recognizing that factors such as O_2 limitation (Spicer and Holbrook, 2007*a*) or transpiration-related CO_2 transport would be disrupted using this method, and therefore gas exchange rates might be somewhat different from that which would be measured *in situ*. Importantly, respiration rates on sampled material remain stable over time, and potential 'wounding' effects from coring are thought to be minimal (Pruyn *et al.*, 2002*b*).

To date, most studies of sapwood or bark respiration consider relatively few species, and rarely consider more than one vegetation type. This is reasonable given that measurements are technically demanding, and that many previous studies have focused on quantifying effects of season, plant age, nutritional status and sampling position. In contrast, here we sought to identify broad trends across a large suite of species and sites. We quantified sapwood, bark and leaf respiration, and relevant tissue properties including N concentration, but also tissue density and bark thickness, as few studies have investigated their effects on stem respiration (but see Bowman *et al.*, 2005). We include a broad range of angiosperm species from three contrasting vegetation types and climate regions in Australia: tropical savanna, tropical rainforest and temperate open forest. Our research questions were as follows. (1) Are there consistent differences between tissues in these physical and physiological properties? (2) What are the scaling relationships between respiration and N across leaves, sapwood and bark? Are the relationships similar for terminal branches and main trunks? (3) Is variation in sapwood and bark respiration also influenced by tissue density and thickness? These properties are implicated in multiple ecological and physiological functions. (4) Across sites that differ in their dominant vegetation types and climates, do we observe similarities in physical and physiological properties, including respiration–N relationships? Are the respiration–N relationships observed in this study distinct or convergent with those reported in a global data compilation (Reich *et al.* 2008)? (5) Which traits, individually or in combination, explain the most variation in branch and trunk respiration?

To the best of our knowledge, Reich *et al.* (2008) have published the only broadscale compilation of respiration data for woody tissues. The focus of that study was on investigating the generality of respiration–N relationships among tissues (roots, stems – including terminal branches and trunks – and leaves) and plant groups (angiosperms and gymnosperms). While the

stem component of that dataset was relatively small (16 species –mostly gymnosperms – and 380 observations), by combining those data with our own we sought to establish a general narrative about the trait drivers of tissue respiration rates.

MATERIALS AND METHODS

Sites, species and tissue selection

We sampled species from three vegetation types/regions in Australia. (1) Temperate open forest (hereafter temperate forest) on low fertility sandstone substrates near Sydney (New South Wales). Data were pooled from two nearby sub-sites: one in Kuring-gai Chase National Park (see Wright *et al.*, 2001 for site details) and another in remnant native vegetation on Macquarie University campus. Mean annual temperature (MAT) is 17.2 °C and mean annual precipitation (MAP) is 1220 mm. (2) Low and high elevation tropical rainforests in northern Queensland (Bradford *et al.*, 2014; Gray *et al.*, 2019). The high elevation sub-sites were located in and around Danbulla National Park (elevation *ca.* 1200 m, MAT 20.4 ° C, MAP 1800 mm; predominantly granite parent material); the low elevation sub-site is near Cape Tribulation [elevation 15 m, MAT 24.3° C, MAP 3500 mm; soil is colluvium from metamorphic origin (Liddell, 2015)]. (3) Tropical savanna on infertile sandy soil in Howard Springs Nature Reserve, 30 km east from Darwin (Wright *et al.*, 2019), MAT 27.6° C, MAP 1736 mm.

Temperate forest species were sampled from February to May 2013, savanna species in June 2013 and tropical rainforest species in May 2014. Between-site differences may thus include contributions from somewhat different mixes of maintenance vs. growth respiration at each site, although our sampling scheme was designed to capture the slower growth period of the year. Expanding on this point, temperate forest species were sampled from late February to May 2013 (autumn); in this region, the peak growth period is spring. Savanna species were sampled in June 2013, in the dry season (peak growth occurs during the wet season, i.e. November–March). Tropical rainforest species were sampled in May 2014, the beginning of the coolest time of year (May–August). At the temperate forest and savanna sites, woody tissues were sampled at a standard height (1 m; 'trunk' data) as well as on terminal branches of a standard diameter (1 cm; 'branch' data). For rainforest species, we sampled trunk data only. Only mature individuals were included. Several study species from temperate forest are shrubs without a single dominant bole; for these, the sampled data were treated as coming from both branches and trunks (see 'Statistical analysis'). In total, we studied 82 species (Supplementary data Appendix 1), sampling 3–8 individuals of most species but 1–2 individuals for the rainforest species *Apodytes brachystylis*, *Citronella smythii*, *Cryptocarya angulata*, *Dysoxylum pettigrewianum* and *Myrsine porosa*.

Branches were cut from plants, re-cut underwater (to minimize gas entering the water column), transported back to the lab with the cut end submerged and stored at 20 °C with the cut end submerged for up to 3 d but discarded if the leaves became wilted (preliminary studies made on temperate forest species indicated sapwood and bark R values were stable for at least 3 d). Trunk material (sapwood and bark) was sampled with a 0.5 cm diameter increment borer (temperate forest) or hammer and chisel (savanna and tropical rainforest). When using the hammer and chisel, the sample was taken to a depth of *ca*. 1 cm from the surface of the sapwood, excluding inner sapwood. When using the increment borer, three cores were collected per individual, these being the same plants for which we collected branch tissue. The cores were collected *ca*. 1 m above the ground and to variable depths, but we used only the outer 1–1.5 cm, excluding any dark heartwood prior to the respiration measurements. Pith was only found in branches, and not all branches contained pith, which was removed when present. In short, all sapwood samples contained only outer sapwood. The sapwood samples were wrapped in damp paper towels, sealed in plastic bags and placed in a cooler with ice for transport back to the laboratory. Bark samples included both inner and outer bark (Romero, 2014), i.e. all tissues outside the vascular cambium, including the secondary phloem, the secondary cortex and the periderm.

Bark and sapwood respiration measurements

Respiration (R_d) was measured on excised portions of bark and sapwood for both branch and trunk. At least 2 h before beginning measurements, *ca*. 15 cm long sections were cut from branches and the ends immediately wrapped in parafilm (Pechiney Plastic Packaging, IL, USA). Bark and sapwood tissues from terminal branches were carefully separated and placed in individual, darkened, translucent chambers at 20 °C to allow CO_2 dissolved in water in the portions of newly exposed tissues to off-gas and the CO_2 efflux to return to equilibrium. As for trunk material, cores were separated into bark and sapwood components and allowed to acclimate in individual chambers at 20 °C for at least 1 h before measurements were made.

R_d of woody tissues was measured using a LI-6400 portable gas exchange system (LI-COR Inc., Lincoln, NE, USA) with custom-built cuvettes made of transparent PVC pipe cut length-wise, with neoprene gaskets affixed to the cut sides and around the interior circumference of the pipe at both ends (Supplementary data Appendix 2). This produced two pieces that could be placed on either side of a branch and sealed together using Velcro straps. An inlet fitting was attached at one end and an outlet fitting at the other to ensure sufficient mixing of air within the cuvette. The sample line of the LI-6400 was cut and the cuvette placed inline such that air in the sample line coming from the console passed through the cuvette before entering the clean, empty and sealed leaf chamber. This allowed us to set the flow to 300–500 μmol s^{-1}, to control the humidity, to use the CO_2 mixer to set the reference CO_2 concentration to 400 ppm and to match the sample and reference IRGAs for each measurement as though we were measuring leaves. To measure branch R_d, cuvettes of either 5 or 7 cm length were attached near the centre of the 15 cm branch, with woody tissue extending on both sides. For measuring trunk R_d, cuvette ends were sealed with clay plugs. The three cores per individual taken from the temperate forest species were combined in the cuvette to generate enough biomass for an accurate respiration measurement, given their small size, whereas this was not necessary with the savanna samples extracted via hammer and chisel.

A fine-wire thermocouple was placed on the surface of the sample in the cuvette, using the leaf thermocouple port on the head of the LI-6400 for continuous monitoring of sample temperature. Air temperature was adjusted to maintain a tissue temperature (T) of *ca*. 20 °C. Where necessary, R_d measurements were standardized to 20 °C (R_{20}) using the equation:

$$R_d = R_{20} Q_{10}^{(T-20)/10} \quad (1)$$

where R_d is measured respiration at a temperature that differed from 20 °C, and Q_{10} was set to 2.2. This Q_{10} temperature was the average value across many measurements reported by Acosta *et al.* (2007). R_d measurements per unit volume of bark and sapwood were converted to a mass basis (nmol g^{-1} s^{-1}) using the densities of bark and sapwood calculated from the volume displacement method (see 'Bark and sapwood properties').

Leaf respiration measurements

Leaf R_d for the savanna species was measured in June 2013, except for the deciduous species *Terminalia ferdinandiana* (leafless at the time), for which R_d data were taken from Eamus and Prichard (1998). Rainforest species were measured in October 2013. For temperate forest species we measured *Callistemon salignus*, *Casuarina glauca*, *Corymbia maculata*, *Eucalyptus racemosa* and *Syncarpai glomulifera* in March 2014 and used data measured by P.B.R. and I.J.W. for *Acacia suaveolens*, *Corymbia gummifera*, *Eucalyptus australasius*, *E. haemostoma*, *Grevillea speciosa*, *Hakea dactyloides*, *H. teretifolia*, *Persoonia levis* and *Phyllota phylicoides* (Wright *et al.*, 2001).

The new measurements of leaf R_d were made using an LI-6400 portable gas exchange system equipped with a broad-leaf cuvette containing a red–blue LED light source. Leaf temperature was set to 25 °C, the standard temperature for leaf R_d, and reference cell CO_2 concentration to 400 ppm for all measurements. Relative humidity ranged between 50 and 75 % with the flow rate adjusted to maintain this range. Leaves were typically measured within 3 h of the branches being excised and were dark-adapted for at least 30 min within the cuvette prior to R_d measurements. Area basis respiration measurements were converted to a dry mass basis (leaf R_d, nmol g^{-1} s^{-1}) via leaf mass per area (LMA), itself calculated from scanned leaves oven-dried at 60–70 °C for a minimum of 5 d. Leaf R_d measurements were standardized to 20 °C using eqn (1), facilitating comparisons across tissues, sites and previously published studies.

Bark and sapwood properties

Tissue density and N concentration were measured on 1 cm long sections of the branch, adjacent to those used for R_d measurements. Section volume (g cm^{-3}) was determined using the water displacement method, then the bark was removed and the volume determined for the sapwood plus pith. Finally, when present, the pith was removed to determine the volume of the sapwood alone, and the volume of the bark was determined by subtracting the intact volume from the sapwood volume. Callipers were used to measure the thickness and diameter of all sapwood and bark samples.

The volume of trunk bark and trunk sapwood samples used for R_d measurements was also determined by water displacement. Samples were dried at 60 °C for 5 d and weighed. Density was calculated as volume per dry mass. Tissue N concentration (N_{mass}, %) was measured using a Leco CHN elemental analyser at The University of Queensland (Appleton Lab).

Statistical analyses

Using the individual replicates, variance components analysis ('vcov' function in the stats R package) indicated that a large portion of the variation in tissue R_d was at the species level (Supplementary data Appendix 3). Site explained 10–25 % of the variation for leaves and sapwood and <1 % of the variation in bark R_d. For branch R_d, the residual variance was larger than that of species and site combined, indicating intraspecific and intra-site variation.

As trait data tended to be right-skewed within many species, species mean values at each site were calculated as geometric means and $\log_{10}$ transformed for use in all subsequent statistical analyses. Because leaf respiration was not measured on the same individuals for which we had branch and trunk data within the temperate forest site, we paired species means of leaf respiration with the individual replicates for sapwood and bark data.

All analyses were carried out in R v. 3.6.1 (R Development Core Team, 2017). Figures were generated using 'ggplot' in the ggplot2 package (Wickham, 2016) unless otherwise specified.

Tissue comparisons

Combining all sites, we tested for differences in R_d, N_{mass} and physical properties across tissues (research question 1), using a nested linear mixed model (LMM) that included random effects of site and species (Supplementary data Appendix 4). We also analysed the data from each site separately, using species as a random factor, to generate pairwise comparisons at the site level. Tukey's pairwise comparisons were carried out using the 'lsmeans' and 'cld' functions in the lsmeans and multcomp packages, respectively. Post-hoc pairwise comparisons of R_d, N_{mass}, density and thickness were not conducted for the temperate forest site because samples were treated as both branch and trunk.

Trait relationships

We tested whether the strength and scaling of R_d–N_{mass} relationships varied across tissues (research question 2) using a combination of ordinary least squares (OLS) linear regression and LMMs, the latter of which accounted for potentially meaningful random effects of species and/or site (Supplementary data Appendix 4). From the LMMs, we extracted the marginal R^2 values corresponding to the fixed effects (Nakagawa and Schielzeth, 2013), as well as the slope coefficients. All trait relationships were visualized using the results of the LMMs rather than the OLS regression although the slope coefficients and R^2 values from the two analyses were similar in most cases (see the Results).

For models that did not meet assumptions of normality (Shapiro–Wilks $P < 0.05$), a visual inspection of the residual plots did not

reveal strong non-linearity or outliers (not shown), therefore we applied a normal distribution to the residual errors. Furthermore, LMMs are largely robust to violations of normality assumptions (Knief and Forstmeier, 2021), as are models with large sample sizes (Ghasemi and Zahediasl, 2012). LMMs were generated using the 'lmer' function in the lme4 package. Fixed effects of all LMMs were evaluated using a Wald test, implemented using the 'Anova' function in the car package. Slope coefficients from LMMs were extracted using the 'emtrends' function in the emmeans package. Marginal R^2 values were extracted using the 'r.squaredGLMM' function in the MuMIn package. Partial residual plots were generated using the visreg package to visualize interactions.

Explaining variation in R_d *using traits*

To further explore the explained variation in R_d, we carried out a type of R^2 decomposition analysis using the OLS regression output. We explored how well N_{mass}, density and thickness explained variation in R_d (research questions 3 and 5) using the R^2 values from the LMM and OLS regression models described above. Then we calculated the relative importance of each predictor and also interactions among predictors using only the OLS output. A relative importance analysis is akin to an R^2 decomposition analysis. In R^2 decomposition, one adds predictors sequentially to a regression model and measures the change in the R^2 with each added predictor. Because the order of predictors strongly influences the R^2, we first decomposed the R^2 values from all possible model orderings and then averaged them to produce a single value (metric 'lmg') for each predictor, adjusted to sum to 1 (Grömping, 2006). One analysis (for bark and sapwood) included N_{mass}, density and their interaction. We then considered branch and trunk bark only, for which we also had tissue thickness in addition to N_{mass} and density. We therefore included N_{mass}, density, thickness and the interaction between density and thickness in that analysis. Relative importance values were calculated using the 'relaimpo' function in the relaimpo R package.

Global comparisons

To visualize Australian species within a global context (research question 4), we superimposed individual replicates from the present study onto the dataset from Reich *et al.* (2008), which contains R_d and N_{mass} for 287 species and 47 locations. The global dataset includes gymnosperms and angiosperms (both herbaceous and woody), and a mixture of field-sampled plants and glasshouse-grown plants (a point we return to in the Discussion). The global dataset includes some leaf data from Australia (140 observations). We compared the Australian and global datasets by testing for differences in R_d–N_{mass} standardized major axis slopes, using the 'sma' function in the smatr R package.

RESULTS

Variation in traits across tissues and sites

When all species and sites were included, R_d, N_{mass}, density and thickness varied strongly with tissue type (Supplementary data Appendix 5). On average, R_d was highest in leaves, intermediate in branches and lowest in trunk tissues (Fig. 1A). Similar to R_d, N_{mass} was highest in leaves and lower in branches and trunks (Fig. 1B). Within branches and trunks, N_{mass} was generally higher in bark than in sapwood (Fig. 1B). Conversely, density was generally lower in bark than in sapwood (Fig. 1C). Trunk bark was generally thicker than branch bark (Fig. 1D). Highly consistent patterns were seen within individual sites (Supplementary data Appendix 6), with the exception that trunk bark and trunk wood did not differ in R_d or N_{mass} for savanna species (Table 1).

Effect of tissue nitrogen concentrations on respiration rates

In general, the slope coefficients from the OLS regression and LMM analyses were similar (Table 1), although in many cases the relationships were stronger when we applied LMMs. Tissue-specific relationships between R_d and N_{mass} were all positive but varied considerably in strength (Table 1). Combining all data, R_d increased with N_{mass} (LMM slope = 0.80, $\chi^2_1 = 171.43$, $P < 0.0001$, Fig. 2A) and the effect was strong (marginal $R^2 = 0.53$). While there was significant heterogeneity among tissue-specific regression slopes (LMM: $\chi^2_4 = 10.83$, $P = 0.03$), they were all less than isometric (log–log slopes < 1; Table 1). For a 10-fold increase in N_{mass}, on average R_d increased most steeply in leaves (*ca.* 4.5-fold increase), followed by bark (*ca.* 2- to 2.8-fold increase), then wood (i. 1.5-fold increase). The effect of N_{mass} on R_d was not statistically significant for sapwood whether we applied OLS or LMM ($P > 0.05$), and the effect of N_{mass} on R_d in stem bark was not significant when using LMM ($P > 0.05$).

There was no evidence that the R_d–N_{mass} relationship varied with site and tissue type simultaneously (a three-way interaction, $P > 0.05$). The R_d–N_{mass} relationship varied strongly with site however (two-way interaction, site × N_{mass}: $P = 0.0008$). For woody tissues alone, the relationships were generally steeper and stronger for temperate forest species than for tropical rainforest or savanna species (Table 1; Fig. 3A). When we included all tissues, the R_d–N_{mass} relationships were more similar to one another and were slightly weaker for temperate forest species (Fig. 3B).

Effects of tissue density and thickness on sapwood and bark respiration

For bark, scaling relationships between density and R_d were similar whether we used OLS or LMM (Table 1). In contrast, relationships between sapwood density and R_d were more strongly negative when we accounted for random effects of site. Bark tissue thickness and R_d were similarly negatively related whether or not we accounted for random effects of site. Branch and trunk R_d generally decreased with increasing tissue density and thickness (Fig. 2B, C; Table 1) and the effect was moderately strong when examining patterns across sites (OLS $R^2 = 0.14$–0.45). R_d–density slopes were nearly isometric (*ca.* |1|) for branch sapwood and trunk bark, and allometric (|>1|) for branch bark, but much less steep for trunk sapwood (|<1|) (Table 1). Fitted slopes indicated that, for a 10-fold increase in density, there was (on average) a 25-fold reduction in

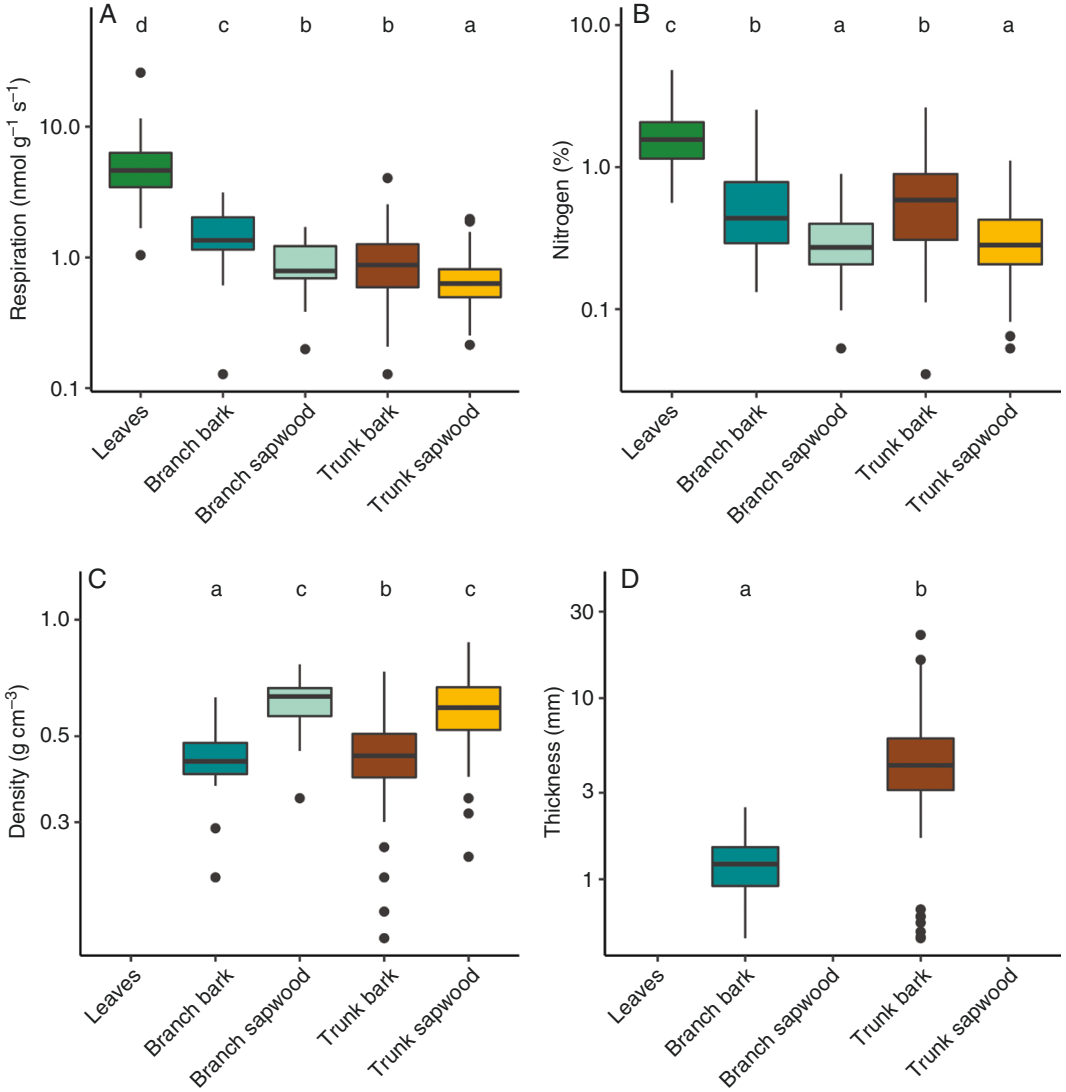

FIG. 1. Variation in (A) mass-based respiration, (B) nitrogen concentration, (C) density and (D) thickness across plant tissues. Lower case letters are *post-hoc* comparisons on mixed effect models that use species–site means, including random effects. Mixed models may detect significant differences between groups that may not be readily apparent from the boxplot.

respiration for branch bark, 10-fold for branch sapwood, 9-fold for trunk bark and 2-fold for trunk sapwood. Slope relationships between bark R_d and thickness were less than isometric (|<1|) for branches and for trunks (Table 1). For a 10-fold increase in thickness, there was a 2.2- to 2.7-fold reduction in respiration. The R_d–density relationship was consistent across tissues and sites (density × tissue × site, $P > 0.05$), as was the R_d–thickness relationship ($P > 0.05$).

Influence of physical properties on R_d–N_{mass} *relationships*

While R_d generally increased with N_{mass}, this was more strongly so (R_d–N_{mass} slopes were steeper) in higher density tissues (bark and sapwood pooled; $\chi^2_1 = 3.94$, $P < 0.05$, Fig. 4A), and less so in thicker compared with thinner bark ($\chi^2_1 = 11.45$, $P = 0.001$, Fig. 4B). For a given N_{mass}, R_d was lower when tissues were thicker or denser. There was no evidence that these two-way interactions (density or thickness × N_{mass}) varied across tissues ($P > 0.05$) or sites ($P > 0.05$).

Explanatory power of traits

Regression approach Tissue density and thickness explained similar amounts of variation in R_d as did N_{mass}, whether considering all woody tissues together or analysing each tissue separately, and whether sites were pooled or analysed separately (Table 1). The chief exception was for species at the savanna site, for which thickness explained more variation (38–59 %) in bark R_d than did N_{mass} (15–50 %).

Including the effects of tissue density alongside N_{mass}, as well as their interaction, notably increased the amount of explained R_d variation (Table 1), whether considering all tissues together or separately. Indeed, this more complex model explained 76% of branch bark R_d variation for temperate forest species based

TABLE 1. *Strength (R^2) and direction (slopes) of relationships between nitrogen concentration (N_{mass}), density (D) and thickness (T), with mass-based respiration rates of bark and sapwood in trunks and branches across three sites ('sites combined') and at each of three sites*

		LMM: random effect of site		OLS: sites combined		OLS: sites analysed separately					
						Savanna		Tropical rainforest		Temperate forest	
Predictor(s)	**Tissue**	**Marginal R^2**	**Slope ± s.e.**	**OLS R^2**	**Slope**	**OLS R^2**	**Slope**	**OLS R^2**	**Slope**	**OLS R^2**	**Slope**
Nitrogen (N_{mass})	**All tissues**	0.53	0.80 ± 0.06	0.50	0.74	0.50	0.81	0.64	0.90	0.43	0.67
	Leaves	0.22	0.58 ± 0.14	0.16	0.43	0.32	0.35	0.38	0.75	0.55	0.70
	Woody tissues	0.19	0.37 ± 0.07	0.15	0.31	0.15	0.32	0.22	0.30	0.28	0.50
	Bark	0.20	0.42 ± 0.12	0.14	0.33	0.16	0.32	0.19	0.37	0.31	0.67
	Branch bark	0.11	0.32 ± 0.29	0.13	0.34	0.004	0.04	–	–	0.30	0.60
	Trunk bark	0.22	0.39 ± 0.13	0.19	0.35	0.14	0.26	0.19	0.37	0.31	0.70
	Sapwood	0.06	0.21 ± 0.11	0.04	0.15	0.06	0.22	0.01	0.06	0.20	0.36
	Branch sapwood	0.02	0.12 ± 0.17	0.09	0.23	0.02	-0.08	–	–	0.09	0.26
	Trunk sapwood	0.05	0.19 ± 0.13	0.03	0.12	0.02	0.15	0.01	0.06	0.34	0.41
Density (D)	**Woody tissues**	0.19	–0.96 ± 0.13	0.16	–0.87	0.19	–1.03	0.22	–0.76	0.24	–1.22
	Bark	0.16	–1.05 ± 0.22	0.17	–1.05	0.11	–1.22	0.19	–0.67	0.27	–1.64
	Branch bark	0.19	–1.39 ± 0.51	0.20	–1.39	0.02	–0.35	–	–	0.45	–2.20
	Trunk bark	0.18	–0.96 ± 0.23	0.18	–0.96	0.19	–1.46	0.19	–0.67	0.21	–1.36
	Sapwood	0.09	–0.72 ± 0.22	0.02	–0.31	0.20	–1.50	0.04	–0.33	0.16	–1.16
	Branch sapwood	0.10	–0.99 ± 0.46	0.12	–0.99	0.14	–0.74	–	–	0.15	–1.15
	Trunk sapwood	0.05	–0.49 ± 0.24	0.02	–0.29	0.03	–0.74	0.04	–0.33	0.19	–1.16
Thickness (T)	**Woody tissues**	0.12	–0.24 ± 0.07	0.12	–0.24	0.59	–0.72	0.09	–0.33	0.004	–0.04
	Branch bark	<0.0001	–0.01 ± 0.25	<0.0001	0.01	0.53	–1.16	–	–	0.01	0.19
	Trunk bark	0.05	–0.17 ± 0.09	0.05	–0.17	0.38	–0.91	0.09	–0.33	0.002	–0.03
$N_{mass} + D + N_{mass} \times D$	**Woody tissues**	0.25	–	0.21	–	0.28	–	0.32	–	0.31	–
	Bark	0.27	–	0.25	–	0.28	–	0.37	–	0.44	–
	Branch bark	0.28	–	0.30	–	0.03	–	–	–	0.76	–
	Trunk bark	0.28	–	0.28	–	0.57	–	0.37	–	0.37	–
	Sapwood	0.14	–	0.04	–	0.21	–	0.09	–	0.24	–
	Branch sapwood	0.10	–	0.16	–	0.20	–	–	–	0.16	–
	Trunk sapwood	0.11	–	0.04	–	0.05	–	0.09	–	0.40	–
$N_{mass} + D + T + D \times T$	**Woody tissues**	0.36	–	0.37	–	0.63	–	0.37	–	0.52	–
	Branch bark	0.33	–	0.37	–	0.54	–	–	–	0.55	–
	Trunk bark	0.35	–	0.36	–	0.61	–	0.37	–	0.53	–

Values shown are either marginal R^2 from linear mixed models (LMMs) or R^2 from ordinary least squares (OLS) linear regression, and slopes are the slope coefficients corresponding to the main/fixed effects. Grey font indicates no significant difference at $\alpha = 0.05$. Note, there were no branch-level data for the tropical rainforest site and no tissue thickness data for sapwood.

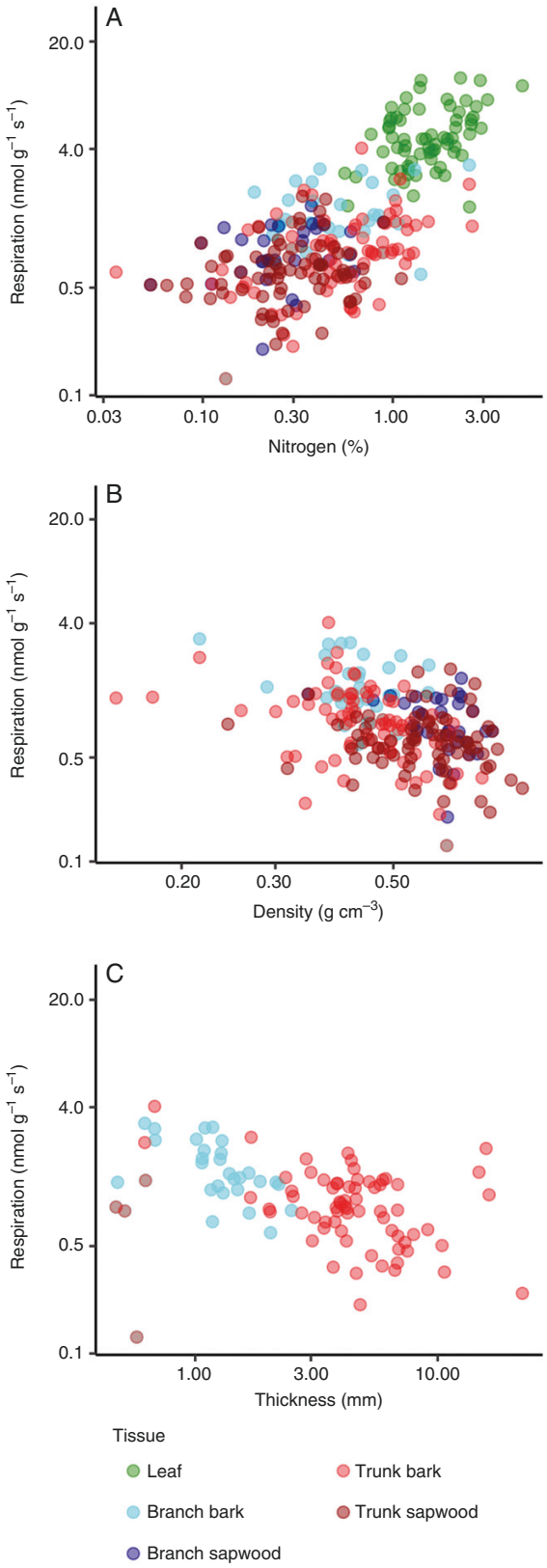

FIG. 2. Relationships between respiration and (A) nitrogen concentration, (B) tissue density and (C) thickness across tissues. Each point represents a species-site mean. For slope relationships, see Table 1.

on the OLS regression. Similarly, including tissue density, thickness and the density × thickness interaction increased the ability to explain variation in bark R_d, whether considering all sites together or separately (OLS R^2 = 0.36–0.63).

Relative importance analyses Decomposing the explained variation in R_d revealed that N_{mass} was not the dominant contributor to the variation in R_d across all tissues. In trunk tissues, N_{mass} contributed slightly more (in sapwood) or a similar proportion (in bark) to the R^2 as did density, whereas in branch tissues density made a larger contribution (Fig. 5A). The N_{mass} × density interaction was also a substantial contributor to the R^2 for branch bark. Considering bark only (for which we also had thickness data), density contributed the most to the R^2 followed by N_{mass} (Fig. 5B). Thickness alone was relatively unimportant in terms of its contribution to the R^2; however, its interactions with tissue density had relative importance values that were similar to, or greater than, those of N_{mass}.

Comparison of Australian data with global data

N_{mass} in our Australian dataset ranged between 0.01 and 4.8 % (Fig. 6A) while the global data extended as high as 5.6 % (Fig. 6B, C). R_d values in the current study were at the lower end of the global range (Fig. 6C), between 0.08 and 27 nmol g^{-1} s^{-1} vs. a maximum of 71 nmol g^{-1} s^{-1} in the global dataset. For the current all-Australian dataset, the all-species and all-tissues R_d–N_{mass} SMA slope [1.18, confidence interval (CI) 1.13–1.24] was similar (Fig. 6C) but significantly flatter than the 'global' slope reported by Reich *et al.* (2008) (1.27, CI 1.24–1.30; test for slope heterogeneity: likelihood ratio$_{d.f.=1}$ = 7.63, P = 0.006). Visual inspection of the 95 % confidence ellipses for the two groups (grey vs. red ellipses in Fig. 6C) suggested that the all-Australian data cloud was shifted both towards the lower left (i.e. to lower N_{mass} and lower R_d) and slightly downwards, towards generally lower R_d at a given N_{mass} (particularly in the range N_{mass} = *ca.* 0.2–2.0 %) and the upper range of R_d in the global dataset was nearly triple that of the present dataset.

DISCUSSION

Here we investigated the controls of bark and sapwood respiration (R_d) in branches and trunks of 82 Australian species, also reporting data for leaves. We demonstrated generality in R_d–N_{mass} relationships within and across tissues, concordant with results from Reich *et al.* (2008) and previous studies. Not previously reported, species with higher density sapwood or bark tended to have lower R_d in these tissues, and bark thickness was negatively related to R_d. Interactive effects were also observed: positive relationships between R_d and N_{mass} weakened as bark and sapwood density increased, and as bark thickness increased. We hypothesize that the effects of tissue density and thickness on respiration are indicative of variation in N pools, with higher density or thicker tissues having a higher fraction of tissue N in structure (i.e. bound within cell walls) and a lower fraction in metabolic pools. Reflecting differences in both N_{mass} and physical properties, respiration rates were significantly higher in leaves than in bark and sapwood, and significantly higher in terminal branches

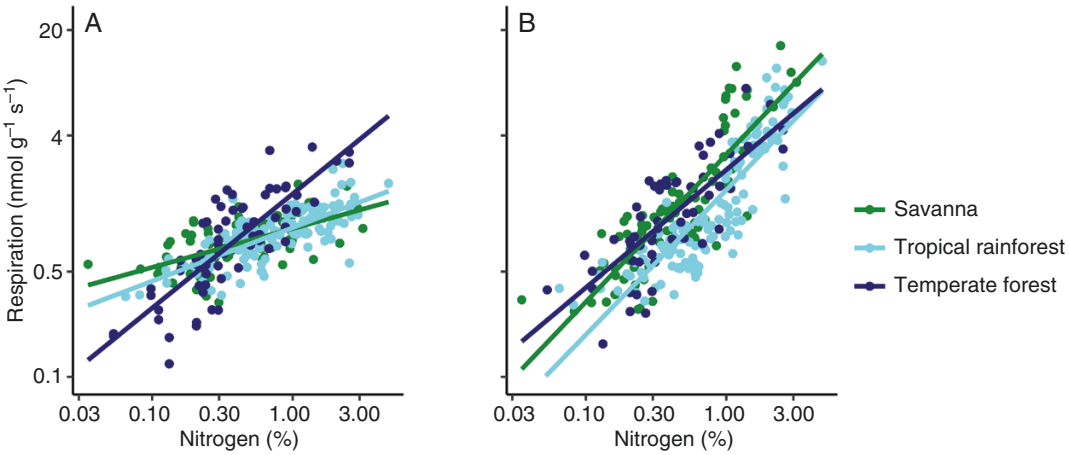

FIG. 3. Relationships between respiration rate and nitrogen concentration across sites, including (A) only woody tissues or (B) leaf, bark and sapwood tissues. Each point represents a species–site mean, and fitted lines represent linear mixed regression models. Axes are logarithmically scaled. For slope relationships, see Table 1.

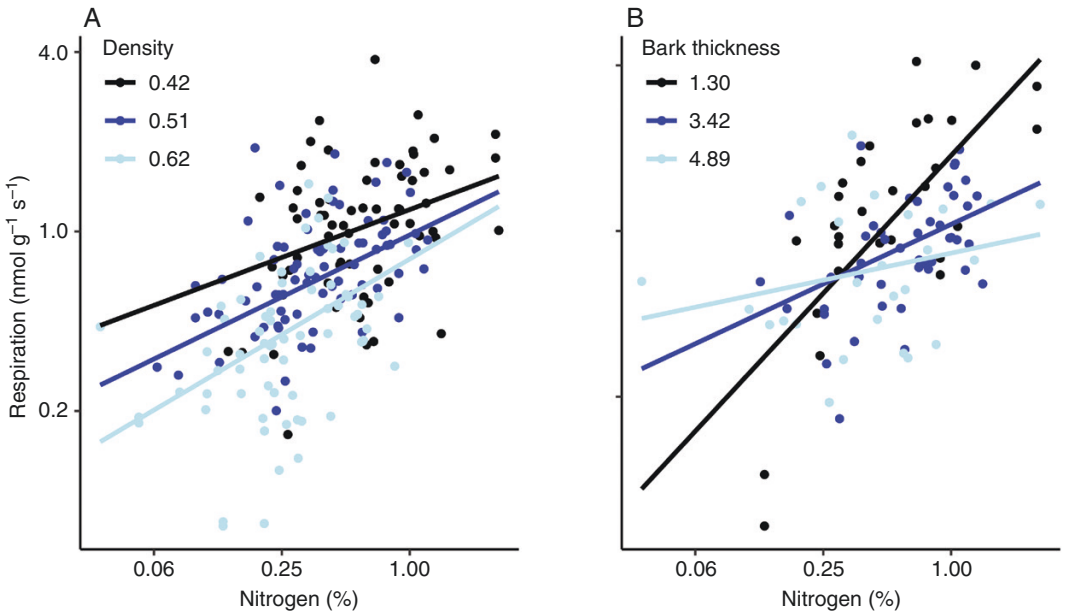

FIG. 4. Partial residual plots to visualize the interaction between nitrogen concentration (%) and each of (A) density (g cm^{-3}) and (B) thickness (mm) on bark and sapwood respiration. Sapwood and bark were included in (A) while only bark is represented in (B). Colours represent different levels of thickness or density (low = black, medium = blue, high = sky blue) and correspond to the 25th, 50th (median) and 75th quartiles, respectively. Study sites have been combined, and each point represents a species–site mean.

than in main trunks. Below we synthesize our findings, placing them in the context of the global study of Reich *et al.* (2008), and discuss the utility of this work for modelling plant respiration.

Variation in nitrogen, respiration and physical properties across tissues

Nitrogen is a fundamental building block of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites (Marschner, 2012). In the present study, N_{mass} was highest in leaves, lowest in branches and trunks, and was consistently higher in bark relative to sapwood (Fig. 1). Patterns of variation in respiration largely reflected those of N, decreasing from leaves to bark to sapwood. The higher N_{mass} and R_d in bark than in sapwood may partially reflect a higher proportion of living cells in bark (Ryan, 1990), but also (and perhaps more so) the fact that the cork cambium is an especially metabolically active tissue zone, and that many woody species have photosynthetically active bark, especially in terminal branches (Pfanz *et al.*, 2002; Rosell *et al.*, 2014; Cernusak and Cheesman, 2015). That seemed to be the case in our dataset: 12 of 15 temperate forest species showed unambiguous evidence of photosynthetic CO_2 refixation in bark (unpubl. data), and most of the savanna species had chlorophyll-containing tissue within the inner bark (Rosell *et al.*, 2015).

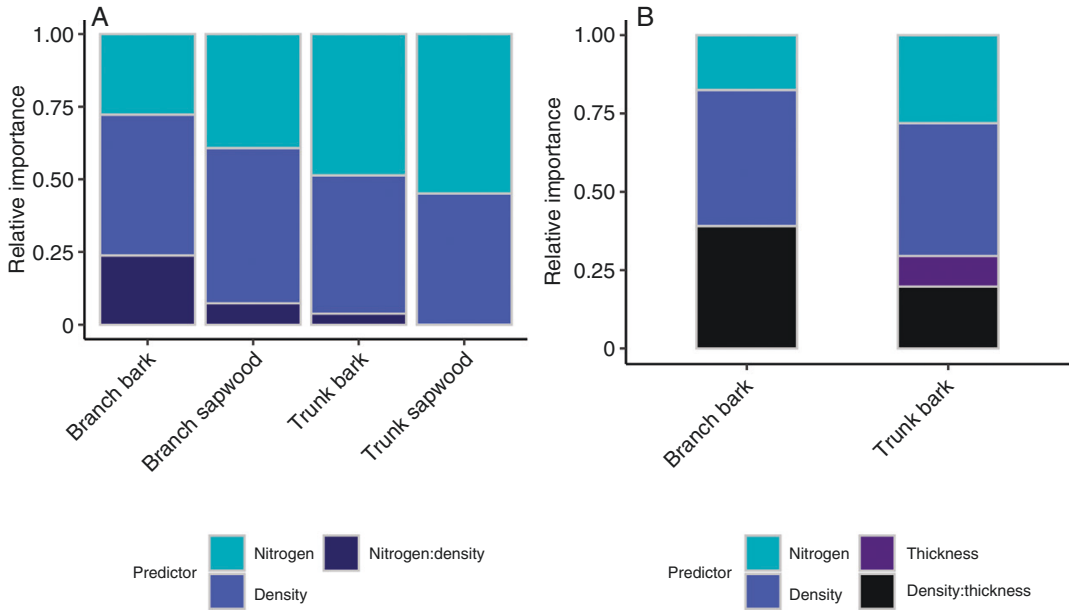

FIG. 5. The relative importance of (A) nitrogen concentration (%), tissue density (g cm^{-3}) and their interaction, and (B) nitrogen concentration, tissue density, tissue thickness (mm) and the interaction between density and thickness, for bark and sapwood tissue respiration. Thickness measurements were only available for bark. Relative importance describes the contributions of individual variables to the R^2 of OLS linear regression models (see Table 1 for R^2 values).

Tissue density was higher in sapwood than in bark. This may reflect distinct functional roles (despite both tissues being derived from the vascular cambium), sapwood playing an important role in plant biomechanics and a higher density reflecting the substantial proportional contribution of fibres [e.g. averaging 45 % of sapwood cross-section in 69 Australian angiosperms (Ziemińska *et al.*, 2015)].

Bark was generally thicker on trunks than on branches, but had similar density, echoing previous reports (Paine *et al.*, 2010; Rosell *et al.*, 2015). In fire-prone ecosystems, such as the savanna and temperate forest studied here, thick outer bark is thought to primarily serve a protective function (Rosell *et al.*, 2014), while thick inner bark enhances storage of water and NSCs (Srivastava, 1964; Rosell, 2016; Rosell *et al.*, 2021).

Controls of respiration

In line with previous studies (see the Introduction), we report positive relationships between N_{mass} and R_d, although the scaling relationships varied among tissues (Table 1). In principle, at a given N_{mass}, a more metabolically active unit of plant tissue should exhibit higher respiration rates, resulting in a steeper R_d–N_{mass} relationship in that tissue. As expected, we observed a steeper R_d–N_{mass} slope in leaves (0.43–0.58) than in bark (0.33–0.44) and sapwood (0.15–0.21; Table 1).

Here, tissue thickness and density were both implicated as exerting some control of R_d and R_d–N_{mass} relationships. Thick cambium and bark may increase resistance to radial diffusion of CO_2, functioning in concert with O_2 limitation to constrain respiration (Steppe *et al.*, 2007) and reduce *in situ* stem efflux rates (Cavaleri *et al.*, 2006). However, here we measured R_d on small, excised tissue samples such that resistance to diffusion is unlikely to explain the observed patterns. We hypothesize that the weaker R_d–N_{mass} relationship in thicker tissues results from a greater proportion of N stored and bound in cell walls [i.e. N found in structural proteins (Bao *et al.*, 1992)], and a lower proportion in physiologically active N pools (e.g. amino acids or soluble proteins). We also detected a lower R_d at a given N_{mass} in higher density tissues, suggesting a higher relative proportion of structural N (vs. metabolic N) therein. As already noted, higher density sapwood typically has a higher fractional contribution from thick-walled fibres. Similarly, higher bark density is positively correlated with mechanical strength, and depends strongly on the presence of thick-walled cells including fibres and sclereids (Chave *et al.*, 2009).

There was no evidence that the R_d–N_{mass} relationship simultaneously varied across tissues and sites (a three-way interaction), despite differences in climate and vegetation type. The relationship between R_d and N_{mass} was positive across all sites, although the slope was strongest for temperate forest species and shallower but similar in savanna and rainforest. Within the temperate forest we sampled a mixture of trees and shrubs (Supplementary data Appendix 1), some with particularly thin bark relative to species at the savanna and rainforest sites. Perhaps a greater proportion of bark N was structural in the savanna and rainforest species, contributing to the shallower R_d–N_{mass} slope observed therein.

Relative explanatory power of traits

Nitrogen is typically considered a primary determinant of respiration (Ryan, 1991; Reich *et al.*, 2008). Our results broadly support that statement, certainly when considering all tissues together, or leaves or bark. Unexpectedly, however, sapwood

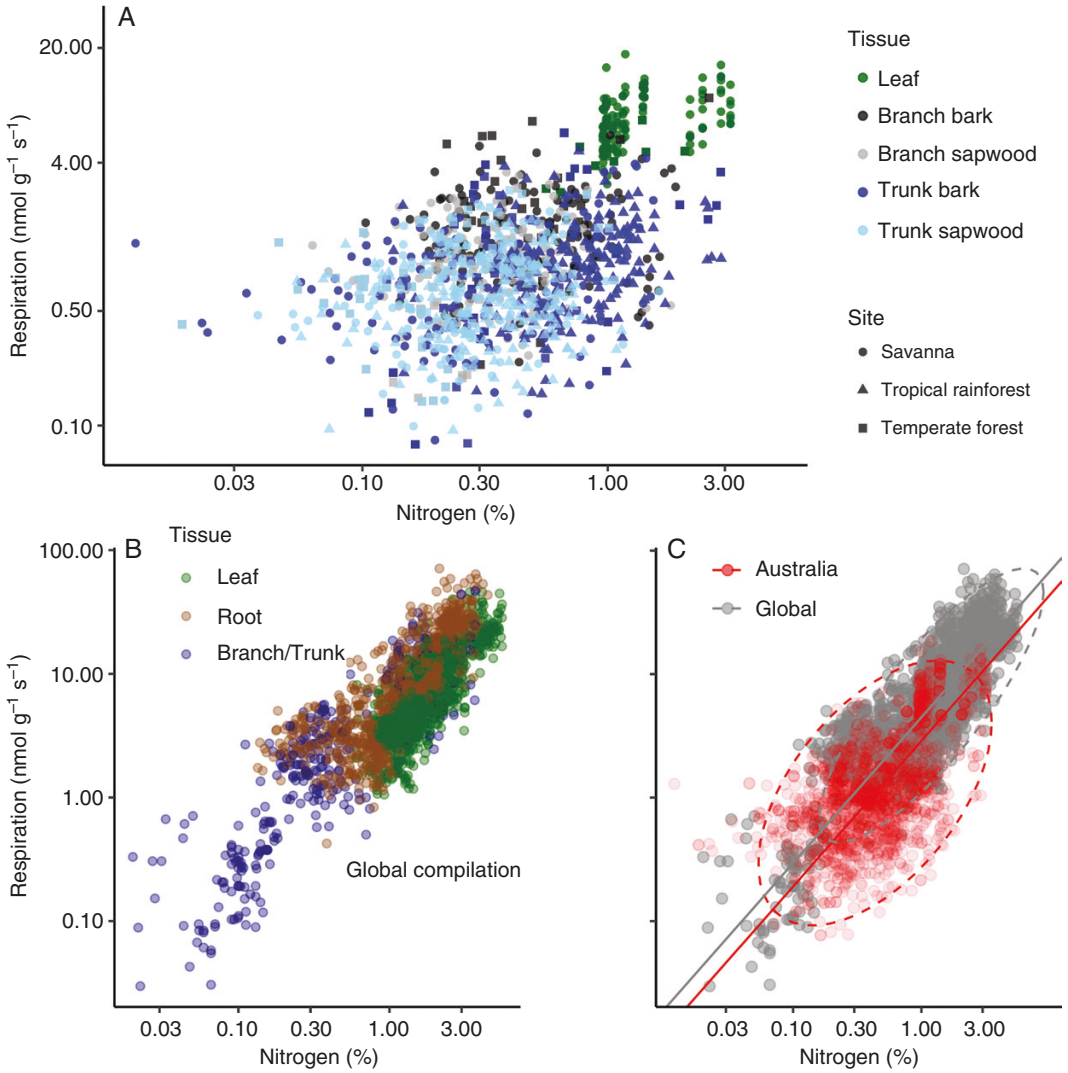

FIG. 6. Variation in respiration with nitrogen concentration, both mass-based, (A) across tissues in the present study. Shapes represent study regions within Australia. (B) Global compilation of respiration vs. nitrogen concentration for leaves, roots, trunk and branch recreated from Reich *et al.* (2008). (C) Comparison of globally compiled data and data from the present study. Each point is an individual plant, and solid lines were generated using the slopes and intercepts of the standardized major axis regression analysis. Dashed lines indicate the 95 % confidence region of each group.

R_d and N_{mass} had a generally weak to negligible relationship when averaged across sites (Table 1). Within woody tissues, N, density and thickness each explained similar amounts of the variation in respiration, and in some cases clearly more so when sites were considered individually (e.g. for temperate forest, N_{mass} of trunk sapwood explained 34 % of R_d; for savanna, bark thickness explained 38 % R_d variation in branches and 53 % in trunks). While it is not surprising that in combination with one another these traits explained more of the variation in stem respiration than they did individually, it is remarkable that up to 76 % of the variation in stem respiration can be explained using only N_{mass} and tissue density (Table 1). However, there were also some cases where these variables explained <10 % of the variation in R_d, and this was particularly true in trunk sapwood considered at individual sites. This may be attributable to radial variation in sapwood properties such as the proportion of living cells, and the activity of those cells. The unexplained variation might also result from varying contributions (across sites or species) of maintenance and growth respiration to measured R_d, despite our sampling being undertaken at times of year with slower growth (see the Materials and Methods).

From the relative importance analyses, N_{mass} contributed the most towards the explained variation for trunk sapwood R_d (on the basis of the R^2), while density emerged as an equally strong, if not more important, contributing variable for the remaining tissues (Fig. 5). Considering bark only, the N_{mass} × density interaction was relatively important for branches but not trunks. When also considering bark thickness, we found the unexpected result that physical properties (and their interactions) were together considerably more important than N_{mass} in terms of their contribution to the R^2.

In sum, our analyses suggest that further consideration of physical properties as well as interactions between N and

physical properties is warranted when investigating trait drivers of R_d variation in woody tissues, particularly for bark.

Global comparisons

Placing our dataset alongside the global compilation of leaf, stem and root data from Reich *et al.* (2008) further demonstrated the broad generality of R_d–N_{mass} relationships across species from diverse biomes, taxonomic groups and tissues. However, the offset between confidence ellipses (Fig. 6C) deserves further comment. In part, the generally lower R_d and N_{mass} in the Australian dataset probably reflects a shift toward 'slower' plant economic strategies, as would be expected for sclerophyllous evergreen species occurring on generally low nutrient soils (Reich *et al.*, 2003; Wright *et al.*, 2004; Reich, 2014). This reasoning also accounts for the cluster of points in the global dataset at the lower range of N_{mass} and R_d; all are gymnosperms and are thus expected to have slower plant economic strategies than angiosperms, and certainly have lower N_{mass} and R_d (Reich *et al.*, 2008). A further difference between datasets is that *ca*. 25 % of rows in the global dataset represent glasshouse-grown plants, and a further 20 % are field-sampled saplings; whereas our new dataset considered field-sampled adult plants only. Quite probably, these differences also drove the offset in the two data clouds.

Future prospects and conclusions

To accurately estimate ecosystem carbon budgets requires reliable measures of autotrophic respiration. In many cases, respiration rates of intact stems are extrapolated to whole plants using scalars such as woody tissue mass, surface area and volume (Meir *et al.*, 2017), and via allometric relationships between branches and trees (Damesin *et al.*, 2002). Achieving sufficient accuracy using such scaled estimates of respiration is constrained by the observation that respiration rates vary across tissue types (Pruyn *et al.*, 2002*a*). We address this knowledge gap, noting that the distribution of above-ground biomass into bark vs. sapwood within stems plays a substantial underlying role in determining rates of stem efflux. It follows then, that including respiration of individual tissues may improve current estimates of stem respiration (Pruyn *et al.*, 2002*b*; Vose and Ryan, 2002). Currently, it is not possible to easily measure respiration rates of individual tissues within intact stems, therefore we opted to excise tissues from intact stems and directly measure their rates of CO_2 efflux alongside their physical properties, recognizing that efflux measured on individual tissues may differ from efflux measured on intact stems. This is because O_2 may be less limiting for respiration than would naturally occur in stems, and because CO_2 efflux is no longer carried away in the transpiration stream, as occurs in intact stems. Nonetheless, this method allows for greater homogenization of temperature throughout the excised tissue, which can typically constrain respiration in intact stems, and wider sampling across a range of species and environments, the latter of which is critical to establish broad generalizations. Future studies should thus carefully weigh up the relative strengths and weaknesses of the chosen methodology.

Using this method, we showed that N_{mass} accounted for considerable variation in R_d across all tissues, with the two traits scaling somewhat more steeply than isometrically when considered across all species and sites (Fig. 6). Our data expand the empirical base behind this general trend, with clear utility for modelling. That said, while N_{mass} accounts for considerable variation in R_d of leaves and bark, this was less the case for sapwood (in contrast to the previous global analysis).

For woody tissues (sapwood and bark), tissue density emerged as an important trait explaining variation in R_d; and for bark, thickness was also important. Interactions among these traits were, in some cases, also important. These findings – that physical properties of tissues explain additional R_d variation alongside N_{mass}, and also seemingly modulate R_d–N_{mass} relationships – are novel in their own right. However, further, they provide potential for refining existing models of autotrophic respiration by adding separate compartments for sapwood vs. bark. For example, the process-based tree stem respiration model, TReSpire (Salomón *et al.*, 2020), includes thickness and density of the outermost tissues (inner and outer bark) and sapwood density (i.e. xylem). A change in the thickness of outer tissues is assumed to be proportional to the stem diameter based on a non-linear allometric relationship and could be further refined to explicitly consider differences between branches and trunks.

Together with the findings of Reich *et al.* (2008), this study represents a significant step forward towards improvement of current ecosystem-scale models, by considering within-stem variation in respiration–N relationships. We include more biomes, angiosperms and species from the southern hemisphere, providing the foundation needed to characterize a fundamental biological relationship. The influence of physical properties, namely tissue density and thickness, is intriguing and only partially understood, motivating the performance of additional studies to investigate its effects on plant physiological traits.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Appendix 1: species list and sampling locations across Australia. Appendix 2: design of sampling apparatus used to measure woody tissue respiration on excised tissues. Appendix 3: variance components analysis for mass-based respiration rates across tissue types. Appendix 4: structures of linear mixed models applied in this study. Appendix 5: main effect of tissue type on mass-based respiration rate, nitrogen concentration, density and thickness from one-way ANOVA. Appendix 6: variation in mass-based respiration, nitrogen concentration, density and thickness for plant tissues across three sites.

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