


RESEARCH ARTICLE

Organic nitrogen improves the water use of tropical tree seedlings cultivated for restoration plantings

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Societal Impact Statement

Restoring degraded tropical lands is important for biodiversity protection and human livelihoods. Newly planted tree seedlings are often challenged by drought brought about by climate change. Here, we explored how nitrogen sources used for plant growth affected the water use of tropical tree seedlings under water limitation. We found that the application of the amino acid arginine reduced water use in the studied seedlings under water limitation, compared with the conventional ammonium nitrate fertiliser. Alternative sources of nitrogen should be considered for tree seedling production in nurseries as this could enhance drought resilience traits and improve the survival of seedlings in restoration plantings.

Summary

- Restoration via tree planting is impacted by climate change-induced water scarcity. Nitrogen (N) supply modulates the morphology and physiology of plants and impacts water use. We compared the responses of rainforest tree seedlings *Acacia mangium* and *Alphitonia petriei* grown with inorganic N (Osmocote™, ammonium nitrate) or organic N (Argrow™, liquid arginine), hypothesising that organic N confers drought resilience by increasing water use efficiency (WUE).
- Seedlings were grown in a glasshouse for 12 weeks with organic or inorganic N in well-watered conditions, and then half the seedlings were subjected to water limitation for a further 4 weeks.
- *A. mangium* grew equally well on all N sources, but water limitation reduced biomass production. In contrast, N sources but not water regimes influenced biomass production in *A. petriei*. Under water limitation, arginine-supplied *A. petriei* had higher WUE and more depleted leaf $\delta^{13}\text{C}$ than inorganic N-supplied plants.
- Our results suggest that organic N in the form of arginine can regulate stomatal conductance in *A. petriei* to convey drought resilience in seedlings. The generality of these findings should be explored to evaluate if organic N is a feasible source for generating drought-resilient seedlings for restoration plantings.

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KEYWORDS

Acacia mangium, *Alphitonia petriei*, ammonium, arginine, nitrate, restoration, water deficit, water use efficiency

1 | INTRODUCTION

Tropical forests and forest restoration projects are impacted by climate change through increased temperatures (Breshears et al., 2021; IPCC, 2018) and drought frequency and severity (Bauman et al., 2022; Chadwick et al., 2016). Improving the survival rate of tropical tree seedlings is key to meeting restoration goals in the United Nation's Decade on Ecosystem Restoration (UN Environment, 2019). The projected increases in temperature and water scarcity are a risk to restoration success unless seedlings are primed for harsh conditions. For example, by planting drought-resilient genotypes and seedlings with enhanced root-to-shoot ratio (Axelsson et al., 2020; Gregorio et al., 2017; O'Brien et al., 2014).

Most research on the effects of drought on the survival of trees focuses on carbon relations and hydraulic safety while discounting the role of nutrients (Gessler et al., 2017; Goldstein et al., 2013) in conferring drought survival or contributing to mortality. Yet, there is evidence that the over-application of nitrogen (N) fertiliser increases the drought sensitivity of tree seedlings by inducing high shoot-to-root ratios, resulting in the inability of seedlings to meet their water demands (Dziedek et al., 2016; Hill & Ex, 2020). Similarly, a recent meta-analysis of trees discovered that N addition increased the diameter of vessels, boosted hydraulic conductance, reduced leaf water potential and induced a higher risk of embolisms (Zhang et al., 2018). These responses suggest that trade-offs between carbon gain and hydraulic function are modulated by N supply, reiterating that over-fertilisation with inorganic N can put plants at increased risk of stress under drought (Zhang et al., 2018). Given these effects of N, generating resilient tropical tree seedlings by manipulating N is in the interest of seedling producers and restoration practitioners. Our study addresses the paucity of knowledge on the ecophysiology of tropical tree seedlings and the interactions between N source and water availability.

Most studies on N and water relations focus on herbaceous plants and compare inorganic N sources (ammonium and nitrate), which can elicit contrasting responses in plant water relations depending on the species (Ding et al., 2018; Tyerman et al., 2017). For example, nitrate supply increased water uptake by 29% compared with ammonium supply in *Phaseolus vulgaris*, an ammonium-sensitive species (Guo et al., 2007). By contrast, water uptake increased approximately twofold in rice plants grown with ammonium compared with nitrate-grown plants, because of higher aquaporin gene expression in the former (Ding et al., 2016). Under water limitation, ammonium nutrition resulted in a ~30% increase in water uptake of rice and a concomitant increase in biomass compared with nitrate nutrition (Li et al., 2009). How N sources affect water use in tree seedlings is not well understood. Ammonium fertilisation increased the shoot hydraulic conductivity of *Pinus taeda* seedlings by ~60% compared with

nitrate supply, with reduced conductivity observed during drought in plants supplied with ammonium (Faustino et al., 2015). Although biomass production was higher with ammonium nutrition, preferred by *P. taeda*, this also translated to a more pronounced growth impairment in drought (Faustino et al., 2015). These examples illustrate that the relationship between N source and water use is likely to impact the resilience of trees, which in turn will affect the survival of tree seedlings in restoration.

In recent years, organic N (e.g., amino acids, peptides and proteins) has received attention as an alternative N source for plants (Cambui et al., 2011; Franklin et al., 2017; Paungfoo-Lonhienne et al., 2012; Soper et al., 2011; Zhang et al., 2019). Organic N can enhance the resilience of tree seedlings through increased root and ectomycorrhizal biomass, improved N use efficiency and drought acclimation (Franklin et al., 2017; Gruffman et al., 2012; Sigala et al., 2020). For example, organic N fertilisation of *Pinus ponderosa* seedlings increased proline concentrations in needles during drought by 80% relative to pre-drought conditions in a glasshouse setting, versus 69% and 27% with nitrate or ammonium, respectively (Sigala et al., 2020). Proline accumulates during abiotic stress and is an osmoprotectant, suggesting that organic N supply may improve drought acclimation in tree seedlings (Sigala et al., 2020; Szabados & Savouré, 2010).

The combined morphological and physiological responses of tropical tree seedlings to N sources and water remain unknown. Yet, understanding this is important to improve restoration outcomes and boost seedling survival rates in degraded landscapes that lack an adequate supply of water and nutrients. Tailoring the N nutrition of seedlings in the nursery to meet specific site conditions could enhance their ability to survive in restoration plantings. We chose the amino acid arginine as the organic N source as it has been used with positive results in studies with boreal trees and N₂-fixing species (Gruffman et al., 2012; Sigala et al., 2020; Zhang et al., 2019) but has yet to be tested on tropical tree species. Specifically, we studied the interaction between N source (inorganic and organic) and water use in two Australian early succession rainforest species (*Acacia mangium* [Willd.]) and *Alphitonia petriei* [Braid & C.T.White]) under controlled glasshouse conditions. *A. mangium* is an N₂-fixing species that improves soil fertility and nutrient cycling of degraded lands and is widely planted in tropical countries for restoration and forest plantations (Koutika & Richardson, 2019). *A. petriei* is a non-fixing species that is used for restoration in Australia (Doust et al., 2008).

We investigated if the N source alters seedling morphological (total biomass, root/shoot ratio, total leaf area, specific leaf area, stem density and nodule dry mass) and physiological traits (xylem water potential, water use efficiency (WUE), leaf $\delta^{13}\text{C}$ ratio and total N uptake) and if this causes distinct patterns in water use when grown with two water supply regimes. We chose a well-watered (80%–90%

water holding capacity) and a mild water deficit treatment (40%–50% water holding capacity) to investigate the physiological responses of seedlings rather than survival mechanisms under severe water limitation. We tested the hypothesis that organic N supply enhances drought resilience via beneficial morphological (i.e., increased stem density and root/shoot ratio and reduced specific leaf area) and physiological traits (i.e., increased WUE) in the studied tree species.

2 | MATERIALS AND METHODS

Seeds of *A. mangium* and *A. petriei* (AustraHort, Cleveland, Queensland) were pre-treated with boiling water and sown into 2-L pots containing a mixture of peat and sand (40/60 v/v) on 11 August 2020. Five nutrient treatments were used, including two inorganic N sources (Osmocote™ Exact 5–6 months [ICL Specialty Fertilisers] and ammonium nitrate), two organic N sources (Argrow® [Arevo] and arginine) and a control (N withheld) (Table 1). Elemental ratios were set by the Osmocote™ treatment, and regardless of N source, all seedlings received a standard rate of nutrients containing 450-mg N, 270-mg P, 360-mg K and 60-mg Mg. These ratios were equalised across N treatments with liquid fertiliser. All treatments received 0.5 g/L of micro-nutrients containing iron (15%), manganese (2.5%), boron (0.2%), copper (1%), molybdenum (0.04%) and zinc (1%) (Micromax, ICL Specialty Fertilisers). In the liquid ammonium nitrate and arginine treatments, fertiliser (100 mL) was applied weekly for 12 weeks from 11 September 2020 and after that bi-weekly for 2 weeks, totalling 16 applications. Depending on the N source, the fertiliser solution was composed of either 10-mM NH₄NO₃ or 5-mM arginine, 5.5-mM KH₂PO₄ and K₂HPO₄, 3-mM K₂SO₄ and 1.5-mM MgSO₄. Fertiliser solutions containing arginine were standardised to pH 5.8–6 with HCl before application. Seedlings were grown in well-watered (90% water holding capacity) conditions for 12 weeks. During this period, each pot was weighed every 2 days to determine water loss and subsequently replenished with deionised water to reach well-watered conditions. On 16 December 2020, water limitation treatment commenced (~45%–50% WHC) and lasted 4 weeks. All pots were weighed every day to maintain moisture conditions according to water treatments. Ten seedlings per species were used in each nutrient and water regime treatment, which totalled 200 seedlings. The study used a randomised complete block design with replicates nested in four separate blocks. Seedlings were grown in a naturally lit glasshouse facility at The University of Queensland from August 2020 to

January 2021. The average temperature in the glasshouse during the study period was 27°C. Seedlings of *A. mangium* were not inoculated with *Rhizobia* for this study but formed nodules.

2.1 | Harvest, measurements and nutrient analysis

Seedlings were harvested from 11 to 14 January 2021, and morphological (total biomass, root/shoot ratio, stem density and specific leaf area) and physiological traits (WUE, leaf δ¹³C ratio and total N uptake) were quantified (Table 2). Xylem water potential readings were taken from cut stems with leaves intact from 9 to 11 a.m. each day using a pressure bomb (Model 3005, Soilmoisture Equipment Corp., USA). Seedlings were separated into leaves, stems and roots. The leaf area of each seedling was measured using a leaf area meter (LI-3100 Area Meter, LI-COR inc., USA). Leaves, stems, roots and nodules were oven-dried at 60°C for 72 h and weighed. Dried leaf, stem and root samples were ground using a ball mill (MM400, Retsch, Germany) and analysed for C/N content and ¹³C isotope signatures at the Chemistry Centre, Department of Environment and Science, Queensland. The standards used for ¹³C follow the International Atomic Energy Agency reference material for laboratories.

TABLE 2 Morphological and physiological traits quantified for *Acacia mangium* and *Alphitonia petriei*.

Measurements	Calculations
Total biomass (g)	Leaves (g) + Stems (g) + Roots (g) + * (+ Nodules (g))
Root/shoot ratio (g/g)	Root (g)/(Leaves (g) + Stems (g))
Stem density (g/cm ³)	Dry weight of main stem (g)/Volume of main stem (cm ³)
Specific leaf area (m ² /kg)	Total leaf area (m ²)/Total leaf dry weight (kg)
Whole plant water use efficiency (g/L)	Total dry mass (g)/Total water used (L)
δ ¹³ C of leaf dry matter	$((^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}} - 1) \times 1000$
Total N uptake (g)	(N concentration (%) × Total dry mass (g)) × 1000

**Acacia mangium* only.

TABLE 1 Nutrient treatments and rates used in the study to cultivate *Acacia mangium* and *Alphitonia petriei*.

	Control (-N) NA	Inorganic N		Organic N	
		Osmocote™ exact 5–6 months	Liquid ammonium nitrate	Argrow® (arginine phosphate)	Liquid arginine
Rates (450-mg N, 270-mg P, 360-mg K, 60-mg Mg)	P, K, Mg	N, P, K, Mg	N, P, K, Mg N supplied weekly	N, P, K, Mg	N, P, K, Mg N supplied weekly

Abbreviation: NA, not applicable.

2.2 | Statistical analysis

We developed linear mixed-effects models, with replicates nested in blocks as the random effects, to determine the impact of N source and water regime on the morphological and physiological traits of *A. mangium* and *A. petriei*. We excluded the control treatment (N withheld) from the analysis as over 80% of seedlings in this treatment failed to survive. Quantile plots were generated from the models to assess the distributions of the residuals for the assumption of normality.

Analysis of variance (ANOVA) was used to determine the significance of each fixed effect (i.e., N and water supply treatment) and the interaction between the N and water treatments in each model. Post hoc Tukey analyses were conducted to determine differences between N sources within a water supply regime (well-watered and water-limited) for each response variable.

All analyses were conducted on R Statistical Computing (version 4.1.3). Mixed-effects models were created using the package 'nlme', post hoc Tukey tests were run using 'emmeans' and graphs were created using 'ggplot2' (R Core Team, 2022).

3 | RESULTS

3.1 | Interaction between N and water on morphological traits

The influence of N source on total plant biomass was unaffected by the water regime in *A. mangium* and *A. petriei* (Figures 1a and S1 and Table 3). Individual effects of N source ($F_{3,61} = 6.05$, $P = .001$) and

water regime ($F_{1,61} = 69.1$, $P < .05$) were observed on the total biomass of *A. mangium*, and an N treatment effect was observed in *A. petriei* ($F_{3,61} = 88.9$, $P < .05$).

In well-watered conditions, the average total biomass of *A. mangium* seedlings was higher ($37.1 \text{ g} \pm 6.1 \text{ SD}$) than in seedlings under water limitation ($27.6 \text{ g} \pm 6$). Within N treatments in well-watered conditions, seedlings that received Argrow® ($40.5 \text{ g} \pm 4.5$) produced more biomass than ammonium nitrate ($34 \text{ g} \pm 6.7$), whereas seedlings grown with Osmocote™ ($38.7 \text{ g} \pm 7.4$) or arginine ($35.1 \text{ g} \pm 3.3$) had similar biomass. In water limitation, seedlings that received Osmocote™ ($32.2 \text{ g} \pm 6.7$) were larger than seedlings that received other N sources (ammonium nitrate, $26.7 \text{ g} \pm 3.1$; Argrow®, $26.9 \text{ g} \pm 6.7$; arginine, $24.7 \text{ g} \pm 2.6$).

Across water regimes, *A. petriei* grown with ammonium nitrate produced the largest average biomass ($28.7 \text{ g} \pm 3.4$). Seedlings that received Osmocote™ ($22.4 \text{ g} \pm 5.3$) and arginine ($24 \text{ g} \pm 3.7$) had intermediate biomass, whereas seedlings grown on Argrow® ($8.07 \text{ g} \pm 5$) had the least biomass.

The root/shoot ratios were influenced by N sources in *A. mangium* ($F_{3,61} = 6.95$, $P < .05$) and *A. petriei* ($F_{3,61} = 6.37$, $P < .05$) (Figures 1b and S1 and Table 3).

Well-watered *A. mangium* seedlings that received Osmocote™ (0.28 ± 0.06) had higher root/shoot ratios compared with seedlings that received ammonium nitrate (0.21 ± 0.09), Argrow® (0.2 ± 0.03) and arginine (0.2 ± 0.04). In water limitation, Osmocote™ (0.27 ± 0.05) seedlings had higher ratios than Argrow® (0.21 ± 0.04) seedlings.

Under well-watered conditions, root/shoot ratios in *A. petriei* seedlings were higher in Argrow® (0.44 ± 0.06) and Osmocote™ (0.43 ± 0.08) compared with ammonium nitrate (0.35 ± 0.05) and

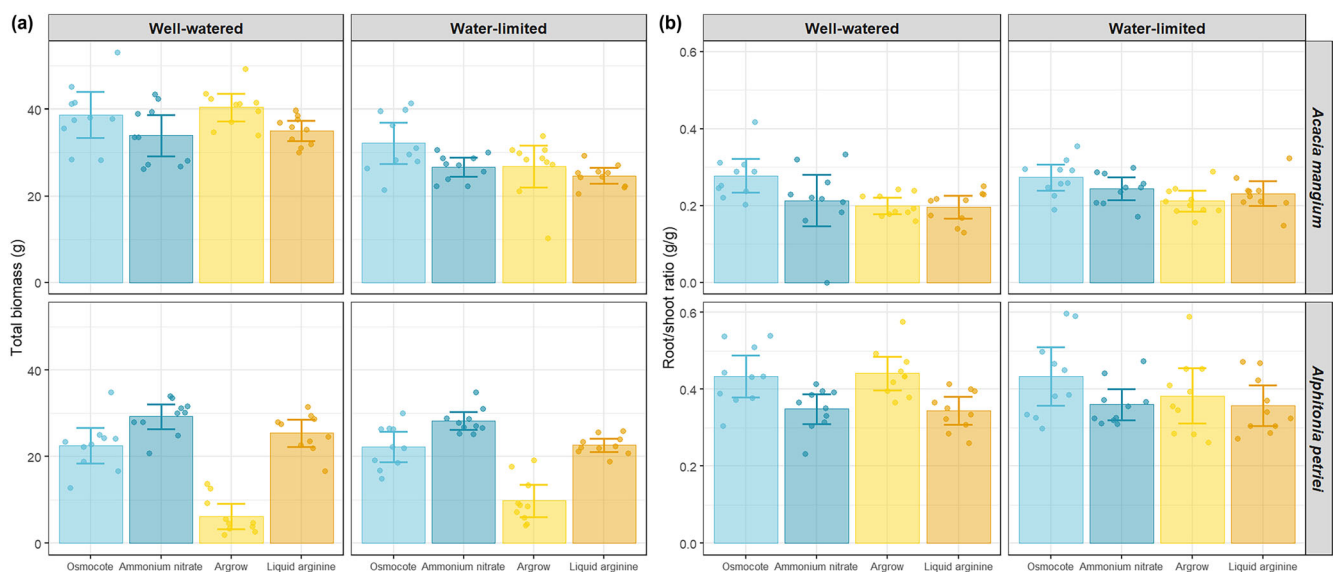


FIGURE 1 (a) Total biomass (g) and (b) root/shoot ratio of *Acacia mangium* (top) and *Alphitonia petriei* (bottom) seedlings supplied with inorganic (Osmocote™, ammonium nitrate) or organic N (Argrow®, liquid arginine) under well-watered and water-limited conditions. All plants were well-watered for 12 weeks before imposing water limitation for 4 weeks. Error bars represent 95% confidence intervals, $n = 10$.

TABLE 3 Results from linear mixed-effects models testing the effects of N treatment, water regime and the interaction between N and water treatments on the morphological and physiological traits of *Acacia mangium* and *Alphitonia petriei* seedlings.

Species and traits	Effect of N treatment	Effect of water regime	Effect of interaction between N treatment and water regime	R ²
<i>Acacia mangium</i>				
Total biomass	$F_{3,61} = 6.05, P = < .05$	$F_{1,61} = 69.1, P = < .05$	$F_{3,61} = 2.19, P = .1$	0.58
Root/shoot ratio	$F_{3,61} = 6.95, P = < .05$	$F_{1,61} = 2.51, P = .12$	$F_{3,61} = 0.63, P = .6$	0.26
Total leaf area	$F_{3,61} = 1.29, P = .29$	$F_{1,61} = 128, P = < .05$	$F_{3,61} = 4.13, P = .01$	0.66
Total N uptake	$F_{3,22} = 27.5, P = < .05$	$F_{1,22} = 48.9, P = < .05$	$F_{3,22} = 9.64, P = < .05$	0.81
Nodule dry mass	$F_{3,60} = 8.95, P = < .05$	$F_{1,60} = 23.7, P = < .05$	$F_{3,60} = 2.01, P = .12$	0.53
Whole-plant water use efficiency	$F_{3,61} = 3.17, P = .03$	$F_{1,61} = 33.8, P = < .05$	$F_{3,61} = 0.92, P = .44$	0.42
Leaf ¹³ C ratio	$F_{3,61} = 0.93, P = .43$	$F_{1,61} = 19.6, P = < .05$	$F_{3,61} = 1.53, P = .22$	0.26
<i>Alphitonia petriei</i>				
Total biomass	$F_{3,61} = 88.9, P = < .05$	$F_{1,61} = 0.04, P = .84$	$F_{3,61} = 2.04, P = .12$	0.78
Root/shoot ratio	$F_{3,61} = 6.37, P = < .05$	$F_{1,61} = 0.24, P = .63$	$F_{3,61} = 1.03, P = .39$	0.25
Total leaf area	$F_{3,61} = 89.6, P = < .05$	$F_{1,61} = 3.39, P = .07$	$F_{3,61} = 3.61, P = .02$	0.79
Total N uptake	$F_{3,22} = 353, P = < .05$	$F_{1,22} = 4.46, P = .05$	$F_{3,22} = 6.74, P = .002$	0.97
Whole-plant water use efficiency	$F_{3,61} = 4.33, P = < .05$	$F_{1,61} = 22.9, P = < .05$	$F_{3,61} = 4.53, P = .006$	0.41
Leaf ¹³ C ratio	$F_{3,61} = 17.0, P = < .05$	$F_{1,61} = 12.6, P = < .05$	$F_{3,61} = 2.50, P = .07$	0.49

Note: Results in the black text represent significant effects ($P = < .05$).

arginine (0.34 ± 0.05). In water limitation, root/shoot ratio did not differ amongst N sources (0.36 – 0.43).

A weak interaction between N source and water supply in the total leaf area was observed in *A. mangium* ($F_{3,61} = 4.13, P = .01$) and *A. petriei* ($F_{3,61} = 3.61, P = .02$) (Figure 2a and Table 3).

Well-watered *A. mangium* had a higher total leaf area ($1989 \text{ cm}^2 \pm 288$) than water-limited seedlings ($1364 \text{ cm}^2 \pm 250$) (Figure 2a and Table 3). In well-watered seedlings, total leaf area was similar between N sources (Argrow[®], $2127 \text{ cm}^2 \pm 186$; arginine, $2031 \text{ cm}^2 \pm 234$; Osmocote[™], $1933 \text{ cm}^2 \pm 348$; ammonium nitrate, $1867 \text{ cm}^2 \pm 326$). In water limitation, seedlings that received Osmocote[™] ($1554 \text{ cm}^2 \pm 301$) had a higher total leaf area than seedlings grown on arginine ($1260 \text{ cm}^2 \pm 117$), whereas the total leaf area was similar in the other N sources (ammonium nitrate, $1355 \text{ cm}^2 \pm 173$; Argrow[®], $1285 \text{ cm}^2 \pm 276$).

Well-watered seedlings of *A. petriei* that received ammonium nitrate ($1792 \text{ cm}^2 \pm 218$) or arginine ($1647 \text{ cm}^2 \pm 317$) had more total leaf area than seedlings that received Osmocote[™] ($1272 \text{ cm}^2 \pm 336$) or Argrow[®] ($395 \text{ cm}^2 \pm 230$) (Figure 2a and Table 3). In water limitation, seedlings that received ammonium nitrate ($1614 \text{ cm}^2 \pm 223$) had the highest total leaf area, whereas arginine ($1361 \text{ cm}^2 \pm 167$) and Osmocote[™] ($1172 \text{ cm}^2 \pm 250$) resulted in an intermediate total leaf area, and Argrow[®] ($611 \text{ cm}^2 \pm 299$) seedlings had the lowest total leaf area.

Shoot water potential, specific leaf area and stem density were influenced by the water regime in both *A. mangium* and *A. petriei* (Figures S2–4). Both species showed reduced shoot water potential

(27.5% and 52.7% decrease in *A. mangium* and *A. petriei*, respectively), reduced specific leaf area (4.63% and 9.6% decrease in *A. mangium* and *A. petriei*, respectively) and increased stem density (18.5% and 7.21% increase in *A. mangium* and *A. petriei*, respectively) under water limitation.

3.2 | Effect of N source and water supply on N uptake

N source and water supply had an interactive effect on the total plant N uptake in *A. mangium* ($F_{3,22} = 9.64, P = < .05$) and *A. petriei* ($F_{3,22} = 6.74, P = < .05$) (Figure 2b and Table 3).

Well-watered *A. mangium* that received Argrow[®] had the highest total N uptake ($768 \text{ mg} \pm 44 \text{ SD}$) followed by arginine ($590 \text{ mg} \pm 54$) and Osmocote[™] ($540 \text{ mg} \pm 28$), whereas seedlings grown on ammonium nitrate ($465 \text{ mg} \pm 64$) had the lowest uptake. In water limitation, Argrow[®] ($542 \text{ mg} \pm 50$)- and Osmocote[™] ($528 \text{ mg} \pm 115$)-grown plants had higher total N uptake compared with ammonium nitrate ($424 \text{ mg} \pm 39$)- and arginine ($390 \text{ mg} \pm 30$)-supplied seedlings.

In well-watered conditions, N uptake was higher in *A. petriei* supplied with ammonium nitrate ($311 \text{ mg} \pm 12$) and arginine ($288 \text{ mg} \pm 13$) than in seedlings given Osmocote[™] ($228 \text{ mg} \pm 21$) and Argrow[®] ($48.6 \text{ mg} \pm 19$). Similarly, in water limitation, seedlings supplied with ammonium nitrate had the highest N uptake ($292 \text{ mg} \pm 9.4$), followed by arginine ($246 \text{ mg} \pm 15$), Osmocote[™] ($211 \text{ mg} \pm 27$) and Argrow[®] ($77 \text{ mg} \pm 19$).

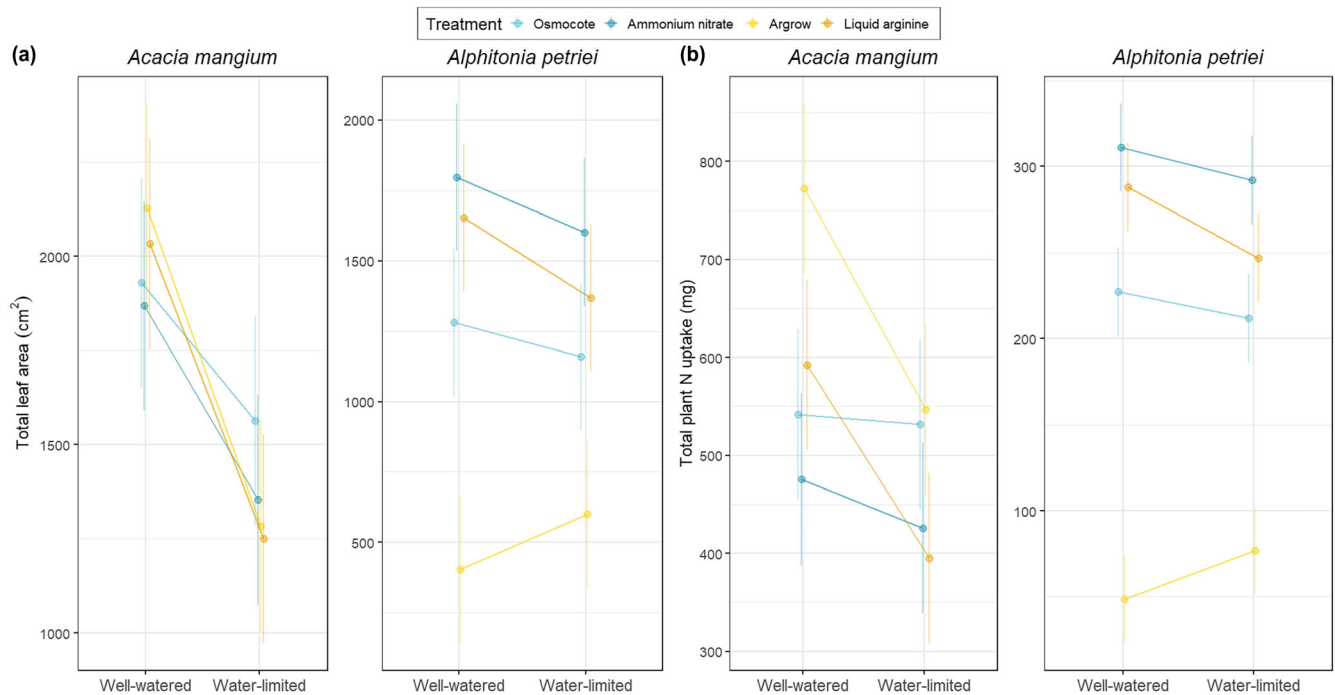


FIGURE 2 (a) Interaction plots for total leaf area (cm²) and (b) total N uptake (mg) of *Acacia mangium* and *Alphitonia petriei* seedlings supplied with inorganic (Osmocote™, ammonium nitrate) or organic N (Argrow®, liquid arginine) under well-watered and water-limited conditions. Error bars represent 95% confidence intervals, $n = 10$ for total leaf area, $n = 5$ for total plant N uptake.

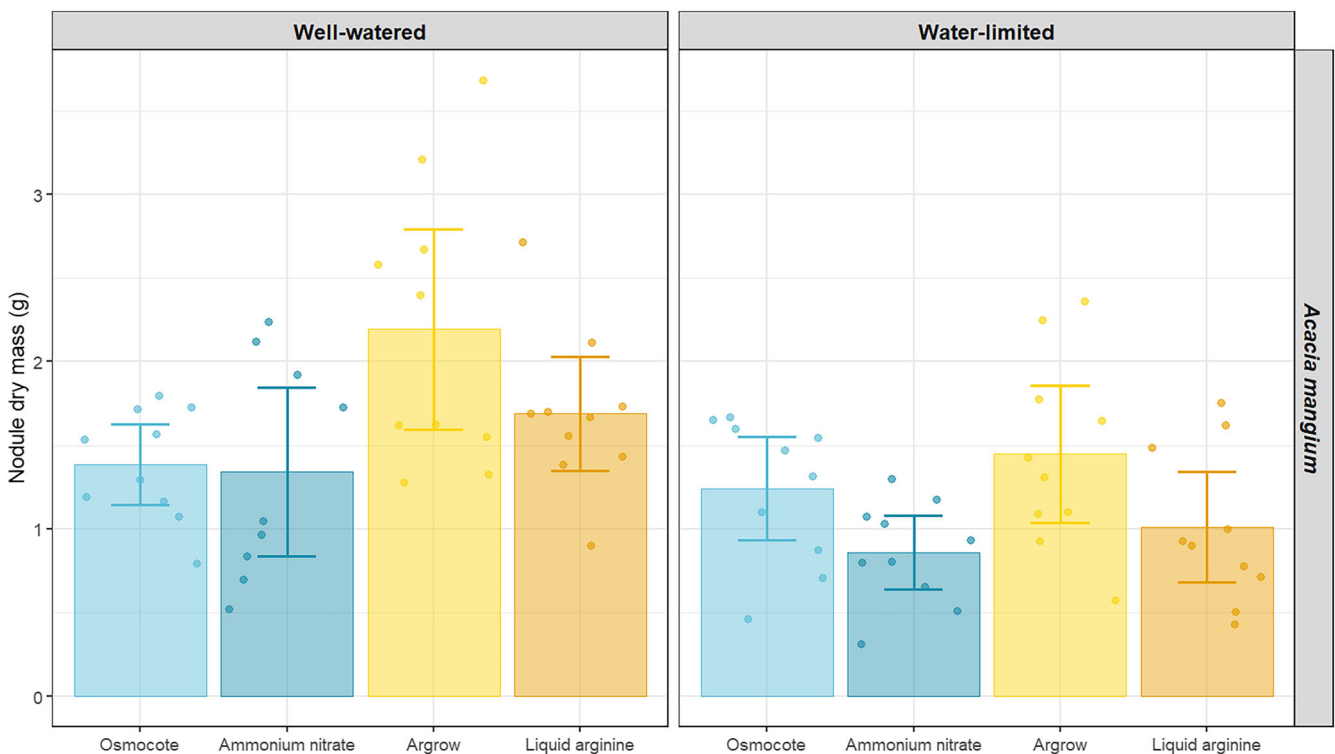


FIGURE 3 Nodule dry mass (g) of *Acacia mangium* seedlings supplied with inorganic (Osmocote™, ammonium nitrate) or organic N (Argrow®, liquid arginine) under well-watered and water-limited conditions. Error bars represent 95% confidence intervals, $n = 10$.

3.3 | Influence of N and water on nodule dry mass in *A. mangium*

Nodule dry mass of *A. mangium* seedlings (Figures 3 and S5 and Table 3) was influenced by N source ($F_{3,60} = 7.87$, $P = < .05$) and water regime ($F_{1,60} = 22.9$, $P = < .05$). Seedlings had a higher average nodule dry mass in the well-watered (1.66 g) than water-limited (1.14 g) treatment.

In well-watered conditions, Argrow[®] (2.19 g) resulted in higher nodule dry mass compared with Osmocote™ (1.39 g) and ammonium nitrate (1.34 g), whereas arginine (1.69 g) treated seedlings had intermediate dry mass. In water limitation, Argrow[®] (1.45 g) treated seedlings had higher nodule dry mass compared with ammonium nitrate (0.86 g) seedlings.

3.4 | N effects on WUE under water limitation

Whole-plant WUE in *A. mangium* (Figure 4 and Table 3) was influenced by N source, albeit weakly ($F_{3,61} = 3.17$, $P = .03$) and water regime ($F_{1,61} = 33.8$, $P = < .05$). Whole-plant WUE was higher in water-limited (5.23 g/L) compared with well-watered (4.5 g/L) *A. mangium* plants.

An interaction between N source and water regime ($F_{3,61} = 4.71$, $P = .005$) on whole-plant WUE was observed in *A. petriei* (Figure 4 and Table 3). In water-limited seedlings, WUE was higher (7.07 g/L) compared with well-watered (5.78 g/L) plants. Seedlings supplied with Argrow[®] (8.1 g/L) and arginine (7.9 g/L) had higher whole-plant WUE

in the water-limited treatments compared with seedlings that received Osmocote™ (5.8 g/L) and ammonium nitrate (6.5 g/L).

3.5 | Relationship between leaf $\delta^{13}\text{C}$, N and water supply

A. mangium had more depleted $\delta^{13}\text{C}$ isotope ratio ($F_{1,61} = 19.6$, $P = < .05$) in water-limited (−30.4 ‰) than in well-watered (−31.5 ‰) conditions (Figures 5 and S6 and Table 3), but effects of N source on leaf $\delta^{13}\text{C}$ ($F_{3,61} = 0.93$, $P = .43$) were not observed.

Leaf $\delta^{13}\text{C}$ isotope ratio of *A. petriei* (Figures 5 and S6 and Table 3) was influenced by the N source ($F_{3,61} = 17.0$, $P = < .05$) and water supply ($F_{1,61} = 12.6$, $P = < .05$). In well-watered conditions, Argrow[®]-supplied plants had less depleted $\delta^{13}\text{C}$ (−28.9 ‰) than the other N treatments (average −27.6 ‰). In the water-limited treatment, Argrow[®] (−28.6 ‰) and Osmocote™ (−27.6 ‰) seedlings had less depleted foliar $\delta^{13}\text{C}$ than ammonium nitrate (−26.9 ‰) and arginine (−26.4 ‰) plants.

4 | DISCUSSION

We studied the relationship between N source (inorganic vs. organic N) and water supply (well-watered vs. water-limited) on the morphological and physiological traits of tropical tree seedlings. The tightly coupled interactions between N, carbon and water impact physiological processes but are often not studied together (Araus et al., 2020;

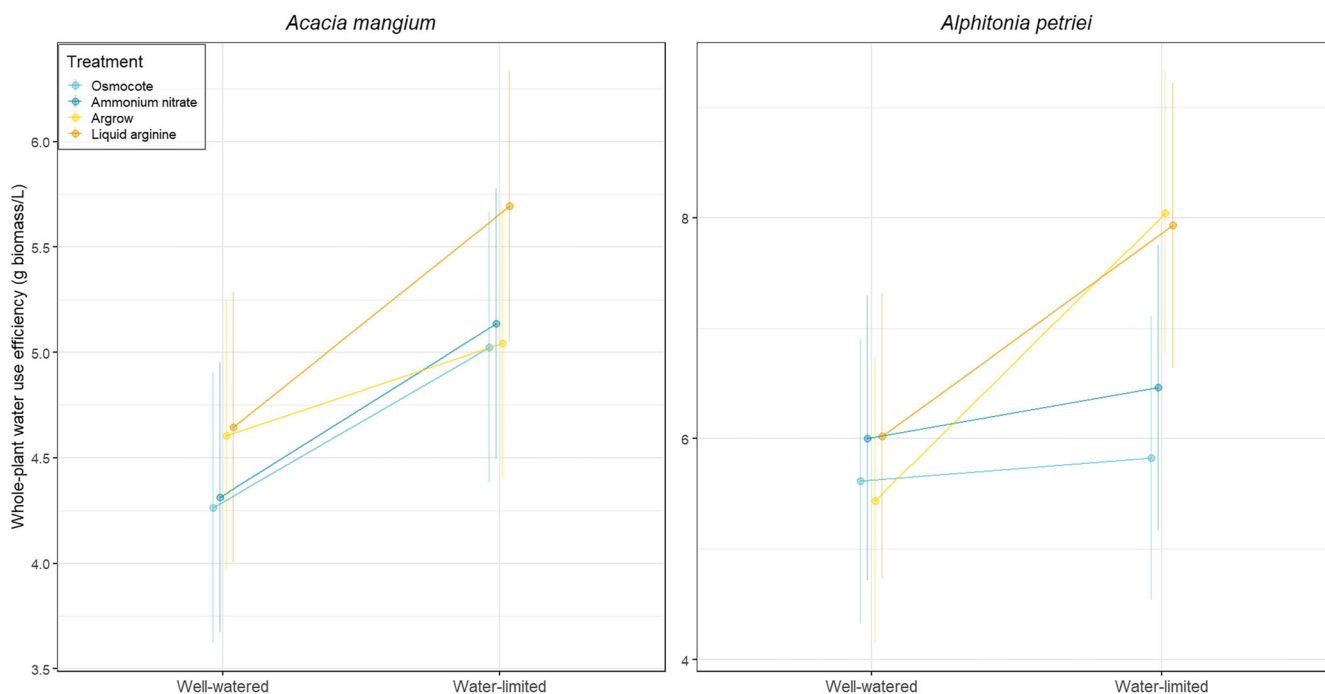


FIGURE 4 Interaction plots for whole-plant water use efficiency (g biomass/L) of *Acacia mangium* (left) and *Alphitonia petriei* (right) seedlings supplied with inorganic (Osmocote™, ammonium nitrate) or organic N (Argrow[®], liquid arginine) under well-watered and water-limited conditions. Error bars represent 95% confidence intervals, $n = 10$.

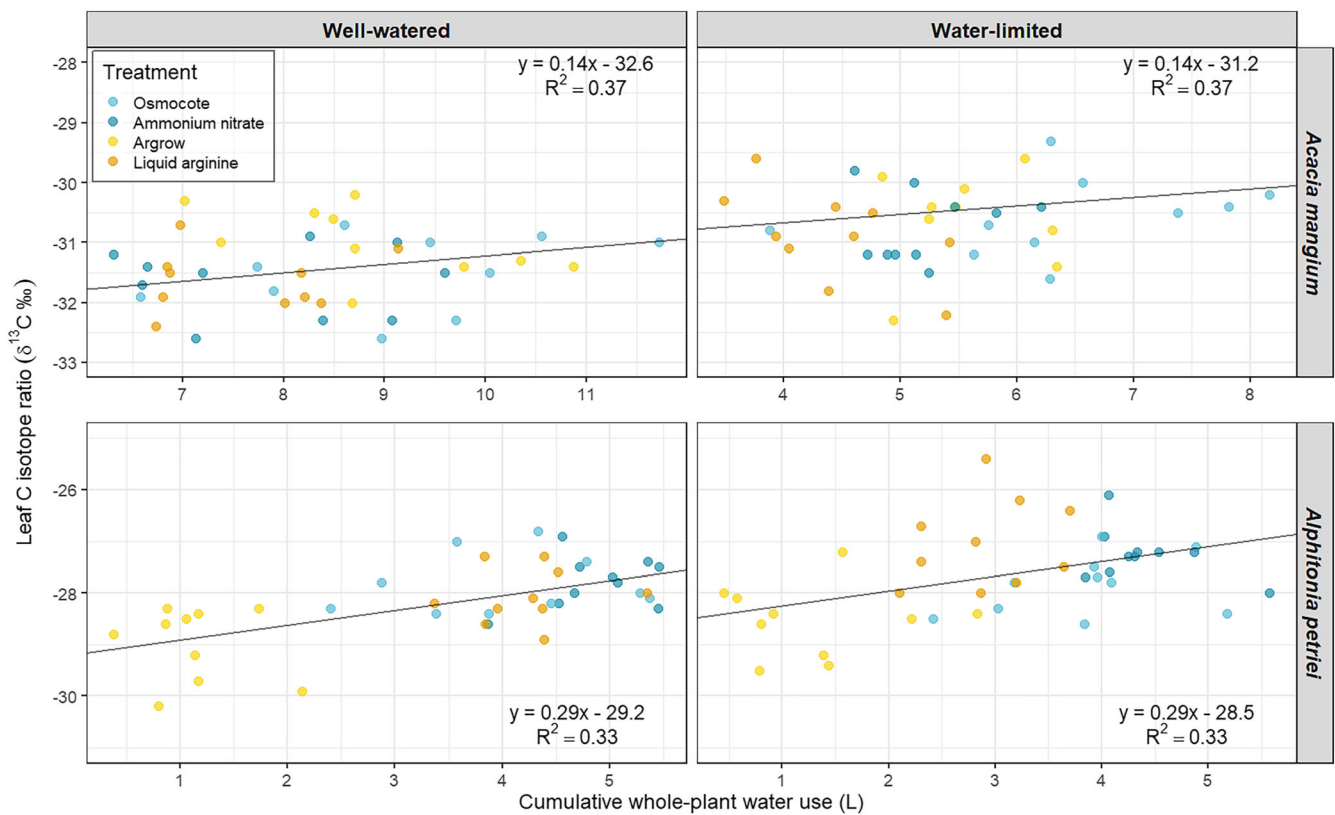


FIGURE 5 Relationship between carbon isotope ratio ($\delta^{13}\text{C}$ ‰) of leaf dry matter and whole-plant cumulative water use (L) of *Acacia mangium* (top) and *Alphitonia petriei* (bottom) seedlings supplied with inorganic (Osmocote™, ammonium nitrate) or organic N (Argrow®, liquid arginine) under well-watered and water-limited conditions. All leaves were used to determine $\delta^{13}\text{C}$, $n = 10$.

Gessler et al., 2017; Salazar-Tortosa et al., 2018). We provide evidence that, in response to N source and water supply, N_2 -fixing *A. mangium* altered its N uptake, whereas non-fixing *A. petriei* improved its WUE. These findings indicate that the applications of organic N could boost the resilience and survival of some species when they are transplanted as seedlings from the nursery to restoration sites with limiting resources for plant growth.

4.1 | Influence of N source and water on morphological traits

N source had minor effects on the biomass accumulation of *A. mangium*, and the growth of seedlings was supplemented by N_2 fixation. The ability to use organic N for growth has been observed in numerous species ranging from crops to trees (Alfosea-Simón et al., 2020; Enggrob et al., 2019; Franklin et al., 2017; Hill & Jones, 2018; Zhang et al., 2019). *A. petriei* showed a preference for liquid ammonium nitrate (inorganic N) as total biomass was up to 23% larger compared with other N sources. Contrary to *A. mangium*, *A. petriei* could not use Argrow® effectively for growth but grew well on liquid arginine, an organic N source, suggesting that slow-release Argrow® is incompatible with this species. Poor growth with Argrow® has been reported in some *Eucalyptus* species (Torgny Näsholm,

Swedish Agricultural University, *pers. comm.* June 2021), but the causalities remain unknown. Microbial competition for amino acids could have exacerbated N depletion (Hill & Jones, 2018) as Argrow® was supplied once at the start of the experiment and liquid arginine was applied regularly. Nonetheless, our results confirm that *A. mangium* and *A. petriei* can use N derived from the supply of organic N.

Reduced water availability had consequential effects on the biomass and leaf area of *A. mangium*, indicating that water limitation impacted the growth rate of seedlings by reducing the investment into leaves for photosynthesis and a corresponding reduction in biomass. This has been corroborated in multiple tree species where water limitation decreases plant performance (Adams et al., 2017). By contrast, *A. petriei* used a conservative growth strategy under water limitation as biomass and leaf area were similar to well-watered seedlings but trait differences (e.g., low shoot water potential, low specific leaf area and high stem density) were observed in water-limited seedlings, indicative of reducing water loss and protecting against embolisms in low moisture conditions similar to other tropical species (Markestijn & Poorter, 2009).

Higher root/shoot ratios have been reported in conifers grown with organic versus inorganic N (Gruffman et al., 2012). However, we did not observe this in the studied species as root/shoot ratios were mostly similar across N sources and water regimes. However, ontogenetic effects and fertiliser availability in the substrate could have

contributed to the minor variation observed in our seedlings (Mašková & Herben, 2018).

4.2 | Advantages of organic N under water limitation

Under water limitation, stomatal conductance was reduced in *A. mangium* as indicated by depleted foliar $\delta^{13}\text{C}$ (Cernusak, 2020), but N sources did not influence WUE or stomatal conductance in this species. Our results contrast with N_2 -fixing *Casuarina equisetifolia* that increased whole-plant WUE and reduced stomatal conductance when supplied with ammonium compared with nitrate in well-watered and drought conditions (Martínez-Carrasco et al., 1998). Further, ammonium-supplied *C. equisetifolia* had higher biomass, which was attributed to an increased rate of carbon assimilation relative to diffusive conductance and hence lower transpiration rates (Cernusak, 2020; Martínez-Carrasco et al., 1998).

Arginine supply increased the WUE of *A. petriei* by 30% and reduced stomatal conductance as indicated by more depleted $\delta^{13}\text{C}$ compared with inorganic N under water limitation. Similarly, *P. ponderosa* seedlings grown with organic N and exposed to drought had increased intrinsic WUE ~14 days sooner compared with seedlings supplied with inorganic N, indicating that organic N may confer resilience under short periods of drought (Sigala et al., 2020). Reduced water use of *A. petriei* supplied with arginine could be linked to indirect effects such as a 17% decrease in the leaf area of water-limited compared with well-watered seedlings. In contrast, lower reductions in leaf area were observed in plants supplied with inorganic N (~9%), suggesting that arginine application more effectively regulates transpiration and WUE in *A. petriei*.

Inorganic N supply in *A. petriei* lowered WUE and potentially exacerbated N-induced transpiration. Generally, soil inorganic N availability concomitantly increases water uptake in plants by increasing root hydraulic conductivity through increased aquaporin expression and results in higher transpiration rates (Araus et al., 2020). High stomatal conductance would be disadvantageous in long periods of water scarcity as larger shoots increase transpiration and water demand (Araus et al., 2020). This could have detrimental effects on seedlings planted at water-limited sites as it may hinder their ability to develop drought avoidance mechanisms under prolonged stress (Claeys & Inzé, 2013).

4.3 | N availability can control water use under water limitation

Under water limitation, *A. petriei* supplied with Argrow[®] had high WUE but stomatal conductance was not reduced. Argrow[®]-supplied seedlings were N deprived as evidenced by low N uptake and biomass compared with seedlings grown on other N sources. This suggests that seedlings were more limited by soil N than water for growth. *A. petriei* potentially decreased root hydraulic conductivity under

water limitation as N, particularly nitrate (after arginine transformation), became less mobile in the soil and more limiting to the plants (Kreuzwieser & Gessler, 2010; Näsholm et al., 2009; Salazar-Tortosa et al., 2018). Although plants tend to decrease stomatal conductance under low N and water conditions (Araus et al., 2020), this could have been viewed as a larger cost to N-deprived Argrow[®] seedlings that elected to maintain transpiration to increase N uptake for stoichiometric balance and physiological processes (Salazar-Tortosa et al., 2018).

4.4 | Effect of N source on total N uptake and nodulation

N was provided via N_2 fixation to *A. mangium* as N uptake was on average up to 31% higher than N supplied across treatments. This was pronounced in well-watered Argrow[®]-supplied seedlings that accumulated ~70% more N than supplied. Limited availability of Argrow[®], and therefore N in the growth substrate, would have promoted nodulation (Ferguson et al., 2019). Investigation is required to determine if arginine promotes nodulation by stimulating nitrogenase activity as observed in nodules of herbaceous legumes inoculated with *Bradyrhizobium diazoefficiens* and *Sinorhizobium meliloti* (Flores-Tinoco et al., 2020). In our study, although the organic N-supplied *A. mangium* seedlings had higher N uptake and nodulation than inorganic N, this did not translate into increased biomass, likely because of the carbon cost of biological N_2 fixation (Sachs et al., 2018). The ability to acquire and store N is however beneficial for seedlings that are planted in degraded sites to overcome N deficiency (Lamb, 2018).

Under water limitation, organic N-supplied *A. mangium* displayed a greater reduction of nodulation and N uptake but maintained higher nodulation than inorganic N-supplied seedlings. Water limitation likely constrained N_2 fixation in all seedlings regardless of the N source because of a reduction in carbohydrate supply with decreased photosynthesis (Parvin et al., 2020). Further, water-limited seedlings potentially downregulated fixation and N export from nodules because of a lower N demand from shoots (Parvin et al., 2020).

In *A. petriei*, total N uptake generally mirrored total biomass in both water regimes with the highest uptake observed in seedlings supplied with ammonium nitrate. High stores of inorganic N could be disadvantageous to plants exposed to drought as stomata may remain open to capture CO_2 for nitrate assimilation (Araus et al., 2020; Guo et al., 2007). Further, excessive N accumulation in plants can increase susceptibility to herbivores (Bobbink et al., 2010). Conversely, seedlings benefit from high N reserves when planted in N-deficient sites to promote field growth (Grossnickle & MacDonald, 2018).

4.5 | Implications for restoration using seedlings grown with organic N

Successful restoration plantings depend on the survival rates of tree seedlings, which are often challenged by limiting soil moisture and

nutrients in degraded land (Gregorio et al., 2017). Here, we have shown that N sources affect the water use and WUE of tropical tree seedlings. This is critical in water-limited environments as plants supplied with inorganic N tended to use more water than their organic N-supplied counterparts. Large-scale nursery production would incur lower costs from reduced water requirements of seedlings grown on organic N. Although we are aware that the use of products like Argrow® and arginine may not be feasible in low- and middle-income countries that lack access to these organic N sources, our research nonetheless shows that organic N sources should be considered where possible for restoration plantings. A cost-benefit analysis could determine if enhanced seedling survival from the use of organic N fertilisers outweighs the cost of its application. Future research should be targeted at the use of other organic N sources especially if they are cost-effective and can be sourced locally.

AUTHOR CONTRIBUTIONS

Vithya Krishnan, Nicole Robinson, Jennifer Firn and Susanne Schmidt designed the experiment. Vithya Krishnan conducted the experiment, analysed the data and led the writing of the manuscript. Nicole Robinson and Susanne Schmidt contributed to data collection. Nicole Robinson and Jennifer Firn assisted with data analysis. Vithya Krishnan, Nicole Robinson, Jennifer Firn, John Herbohn and Susanne Schmidt contributed to the writing and revision of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study are available upon request from the corresponding author.

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SUPPORTING INFORMATION

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

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