

**Responses to competition for nitrogen between subtropical native tree seedlings and exotic grasses are species-specific and mediated by soil N availability**

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3 1 **Responses to competition for nitrogen between subtropical native tree**  
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5 2 **seedlings and exotic grasses are species-specific and mediated by soil N**  
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8 3 **availability**  
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38 17 **Keywords:** ammonium, interspecific competition, net nitrogen uptake capacity, nitrogen  
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40 18 acquisition, nitrate, organic nitrogen, reforestation, subtropics  
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45 20 **Running head:** Soil N mediates tree-grass competition  
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3 21 **Abstract**  
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5 22 Competitive interactions between native tree seedlings and exotic grasses frequently  
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7 23 hinder forest restoration. We investigated the consequences of competition with exotic grasses  
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9 24 on the growth and net nitrogen (N) uptake capacity of native rainforest seedlings used for  
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11 25 reforestation depending on soil N availability and N source. Tree seedlings and grasses were  
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13 26 grown in the greenhouse in different competition regimes (one tree species vs. one grass  
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15 27 species) and controls (grass monocultures or single tree seedlings) at low and high soil N.  
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17 28 After eight weeks, we quantified net N uptake capacity using <sup>15</sup>N-labelled organic (i.e.  
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19 29 glutamine and arginine) and inorganic (i.e. ammonium and nitrate) N sources and biomass  
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21 30 indices. Depending on soil N availability, we observed different species-specific responses to  
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23 31 growth and N acquisition. Tree seedlings generally increased their net N uptake capacity in  
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25 32 response to competition with grasses, although overall seedling growth was unaffected. In  
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27 33 contrast, the responses to competition by the grasses were species-specific and varied with  
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29 34 soil N availability. The different N acquisition strategies suggest the avoidance of competition  
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31 35 for N between trees and grasses. Overall, the results highlight that quantifying underlying  
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33 36 mechanisms of N acquisition complements the information on biomass allocation as a  
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35 37 measure of responses to competition, particularly with varying environmental conditions.  
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## 38 **Introduction**

39 Plant-plant interactions shape the structure of plant communities (Callaway and  
40 Walker 1997, Brooker 2006, Tylianakis et al. 2008). Their magnitude and direction are  
41 influenced by factors including life stage, physiological requirements as well as  
42 environmental conditions (Callaway and Walker 1997). The interplay between these biotic  
43 and abiotic factors determines the outcome of species interactions which in turn affects the  
44 coexistence and spatial distribution of species (Brooker et al. 2008, Soliveres and Maestre  
45 2014). A main interaction is the competition for resources such as soil nutrients. The ability of  
46 plants to acquire nutrients is determined by morphological and physiological traits, such as  
47 uptake rates of nutrients (Casper and Jackson 1997). By modifying nutrient uptake in  
48 response to environmental cues, such as nutrient availability, plants can be more competitive  
49 but the extent of such response varies (Hodge 2004).

50  
51 Competitive interactions between native and exotic plant species are relevant in the  
52 context of global environmental change, because there is evidence that the predicted changes  
53 in the abiotic environment will likely influence competition in favor of exotic species to  
54 (Bradley et al. 2010, Verlinden et al. 2014). The superior competitive ability of exotic plant  
55 species is frequently a key factor promoting invasions, with exotic species able to better  
56 exploit limited resources, to the detriment of native species (D'Antonio and Vitousek 1992,  
57 Gioria and Osborne 2014). Changing nutrient status, such as increased nitrogen (N)  
58 availability with atmospheric deposition, can alter plant growth and biomass allocation in a  
59 species-specific manner, and consequently results in shifts in the outcome of interspecific  
60 competition (Rennenberg et al. 2009). Here, we investigate the competition between exotic  
61 and native plants comparing the response strategies of different species, and how resource  
62 availability modulates these interactions. A focus on N physiology is warranted because N is  
63 quantitatively the most important of soil-acquired nutrients (Marshner 1995), limits plant

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3 64 growth in most ecosystems (Agren et al. 2012), drives competition (Eller and Oliveira 2017),  
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5 65 and N availability and uptake can vary considerably in response to environmental change  
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7 66 (BassiriRad 2000, Kuster et al. 2016). Since most studies focus on biomass production as  
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9 67 indicator of the magnitude and direction of competitive interactions, even though other factors  
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11 68 besides competition may influence biomass, (Trinder et al. 2013), we also examined net N  
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13 69 uptake as a process that underlies the interaction between native and exotic species, because  
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15 70 exotic species might alter soil N availability, show strong preferences to certain N forms  
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17 71 and/or interfere with the N acquisition of native species, therefore affecting their performance  
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20 72 (Fraterrigo et al. 2011, Huangfu et al. 2016, Eller and Oliveira 2017).  
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24 74 While earlier studies have focused mainly on the acquisition of inorganic N, organic N  
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26 75 uptake has received increasing attention due to its potential to drive niche differentiation and  
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28 76 species coexistence, and therefore ecosystem stability (Schmidt and Stewart 1999, McKane et  
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30 77 al. 2002, Kielland et al. 2006, Näsholm et al. 2009, Ashton et al. 2010). A plant's ability to  
31  
32 78 use a wider array of N sources can result in competition avoidance (e.g. Miller et al. 2007,  
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34 79 Simon et al. 2010, Simon et al. 2014, Li et al. 2015). Studies that have quantified the uptake  
35  
36 80 of organic and inorganic N forms found species-specific preferences for different N forms  
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38 81 (e.g. Persson et al. 2003, Weigelt et al. 2005, Simon et al. 2010, Li et al. 2015). For example,  
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40 82 European beech (*Fagus sylvatica*) shows a preference for organic rather than inorganic N,  
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42 83 whereas the opposite is true for sycamore maple (*Acer pseudoplatanus*) (Li et al. 2015). In a  
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44 84 study on excised roots of native and exotic grasses of Australian savannas, a general  
45  
46 85 preference for ammonium was found, but preferences for nitrate and the amino acid glycine  
47  
48 86 were species-specific (Rossiter-Rachor et al. 2009). Furthermore, the uptake capacity of  
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50 87 different N sources is influenced by interspecific competition (e.g. Miller et al. 2007, Simon  
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52 88 et al. 2010, Simon et al. 2014, Li et al. 2015). For example, when grown in competition with  
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54 89 sycamore maple, the capacity for organic N uptake of European beech seedlings was reduced  
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3 90 (Simon et al. 2014). However, the responses of competition with regard to N acquisition are  
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5 91 also influenced by environmental factors (Simon et al. 2014, Li et al. 2015). Overall, most  
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7 92 studies that have examined the acquisition of different N forms in context of competition have  
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9 93 focused either on herbaceous or woody species in temperate ecosystems, rather than the  
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11 94 interactions between trees and grasses. These, however, are important in the context of  
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13 95 conservation and ecosystem restoration, because the successful establishment of tree seedlings  
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15 96 (commonly planted in order to restore a plant community) often depends on the outcome of  
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17 97 their interaction with grasses (Erskine et al. 2005, Doust et al. 2008, Elgar et al. 2014), where  
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19 98 N is likely to play a determinant role (Coll et al. 2004, Barbosa et al. 2014).  
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24 100 Tree-grass interactions have been broadly studied in savanna ecosystems measuring  
25  
26 101 the influence of abiotic factors (e.g. light, water and nutrient availability) that mediate the  
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28 102 interaction (e.g. Ludwig et al. 2001, van der Waal et al. 2009, Moustakas et al. 2013) and in  
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30 103 studies investigating the long-term coexistence of these contrasting life forms (e.g. Jeltsch et  
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32 104 al. 2000, Sankaran et al. 2004). With regard to forest regeneration, tree-grass interactions have  
33  
34 105 been studied in the context of secondary succession, tree regeneration in forest gaps after  
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36 106 disturbances, or meadows (e.g. Coll et al. 2004, Bloor et al. 2008a, Elgar et al. 2014), where  
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38 107 tree seedlings interact with the already established plant community commonly composed of  
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40 108 early successional life forms, including grasses. However, research on the effect of  
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42 109 herbaceous vegetation on tree seedlings mainly focusses on seedling survival and growth (e.g.  
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44 110 Davis et al. 1998, Bloor et al. 2008b, Meli and Dirzo 2013), physiological aspects such as  
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46 111 water relations (e.g. Quinteros et al. 2017) or photosynthesis (e.g. Davis et al. 1999, Loik and  
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48 112 Holl 2001), while competition for nutrients, specifically N, has received little attention  
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50 113 despite the potential of belowground competition with grasses to influence tree growth and  
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52 114 establishment (e.g. Cramer et al. 2010) and the influence of soil N availability on the  
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54 115 competition outcome (e.g. Cramer et al. 2012).  
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5 117 Here, we studied the interactions between native tree seedlings and exotic grasses  
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7 118 because ecological forest restoration efforts are frequently hindered by competition from  
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9 119 exotic grasses (Kanowski et al. 2003, Erskine et al. 2005). In reforestation programs, grasses  
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11 120 successfully establish even after the planting of seedlings without regular weeding. To mimic  
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13 121 these conditions, we used nursery-grown tree seedlings and grasses grown from seed in our  
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15 122 study. We selected tree species that are relevant in the context of restoration of riparian zones  
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17 123 in subtropical Australia (Catteral and Harrison 2006, Gageler et al. 2014), including an N<sub>2</sub>  
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19 124 fixer. Tree species with the capacity to fix atmospheric N<sub>2</sub> via symbiotic rhizobacteria in root  
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21 125 nodules are commonly used in restoration programs with the intent to improve the overall N  
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23 126 supply at a site (Erskine et al. 2005). Their N<sub>2</sub> fixing ability would make them largely  
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25 127 independent from external soil N supply (Messina and Barton 1985) and potentially alleviate  
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27 128 competition for N (Epron et al. 2006). We conducted a greenhouse experiment to advance the  
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29 129 understanding of whether competition for N plays a role in the interaction between seedlings  
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31 130 of two native tree species and two exotic grass species, and the potential effects of soil N  
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33 131 availability. Our objectives were to (i) determine the effect of interspecific competition on  
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35 132 inorganic and organic N acquisition and biomass indices as response strategies of tree  
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37 133 seedlings and grasses, and (ii) evaluate the impact of low *versus* high soil N availability on the  
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39 134 competition outcome in terms of N acquisition. We hypothesized that tree seedlings and  
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41 135 grasses potentially avoid competition by using different N forms (i.e., fast-growing grasses  
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43 136 using inorganic forms and relatively slow-growing trees using organic forms), that growth and  
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45 137 net N uptake capacity of tree seedlings is negatively affected in the presence of competing  
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47 138 exotic grasses, and that this negative effect is lessened at high soil N availability.  
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55 140 **Materials and Methods**56  
57 141 *Study species and plant material*  
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3 142 We selected two native tree species used in riparian reforestation in the Maleny region  
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5 143 (26.7° S, 152.8° E, Queensland, Australia), angiosperm *Acacia melanoxylon* R.Br (Fabaceae)  
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7 144 and gymnosperm *Podocarpus elatus* R.Br. ex Endl. (Podocarpaceae). *A. melanoxylon* is an  
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9 145 early successional, N<sub>2</sub> fixing species native to south-east Australia (Smith et al. 2008,  
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11 146 Birnbaum et al. 2012) that occurs in rainforests and wet sclerophyll forests (Doran and  
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13 147 Turnbull 1997). *P. elatus* is a late-successional endemic species that grows in subtropical  
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15 148 rainforests (Smith et al. 2008). Seedlings of *A. melanoxylon* (5 months old, ~ 40 cm tall) and  
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17 149 *P. elatus* (10 months old, ~ 30 cm tall) were obtained from a local nursery (Brush Turkey  
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19 150 Enterprises, Maleny, Australia). We selected tree species with contrasting successional status  
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21 151 to explore potential differences in their N acquisition strategies related to growth rate (Li et al.  
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23 152 2015), and species adaptation to N forms available in soils of different successional status  
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25 153 (e.g. higher nitrate availability in early successional sites compared to late sites) (Kronzucker  
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27 154 et al. 1997, Britto and Kronzucker 2013). The exotic grasses *Pennisetum clandestinum*  
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29 155 Hochst. ex Chiov. (Poaceae) and *Cynodon dactylon* (L.) Pers. (Poaceae) are perennial,  
30  
31 156 originate from Africa and have been introduced to tropical areas around the world (Judd  
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33 157 1979). Spreading via rhizomes, stolons and seeds, these grasses naturally occur in grasslands  
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35 158 and are common in pastures and lawns in their introduced range (Judd 1979). In subtropical  
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37 159 Australia, these two grass species are reported as a threat to restoration efforts, and their  
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39 160 negative effects on the growth of native tree seedlings have been observed in the field (M.  
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41 161 Amos, pers. comm.). Grass seeds were purchased from Royston Petrie Seeds (Mudgee,  
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43 162 Australia). From here on, species will be referred to by their genus, i.e. *Acacia*, *Podocarpus*,  
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45 163 *Pennisetum* and *Cynodon*.  
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#### 165 *Experimental setup*

166 Tree seedlings were planted and grasses were sown from seed into 1 L pots (125 mm  
167 diameter, Anova Solutions, Brisbane, Australia) with a 1:1 mixture of grade A pit sand (grain

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3 168 size: 0.5 mm) and G2 vermiculite (grain size: 1-2 mm) in interspecific competition or under  
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5 169 control conditions at low and high soil N availability. Competition treatments consisted of one  
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7 170 tree individual surrounded by 10-12 individual grasses that were uniformly distributed ~4 cm  
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9 171 from the seedling stem. The four species combinations were *Acacia* vs. *Pennisetum*, *Acacia*  
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11 172 vs. *Cynodon*, *Podocarpus* vs. *Pennisetum* and *Podocarpus* vs. *Cynodon*. Control treatments  
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13 173 consisted either of a single tree seedling or 12 individuals of one grass species (i.e.  
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15 174 monoculture). Tree seedlings were not large enough to result in shading for the grasses (A.  
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17 175 Bueno, *pers. obs.*). Because competition for N is highest under conditions of N limitation  
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19 176 (Wilson and Tilman 1991, Casper and Jackson 1997), all competition regimes received the  
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21 177 low soil N treatment. To ensure sufficient replication, the high soil N treatment was only  
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23 178 applied for tree species vs. *Pennisetum* because *Cynodon* had poor germination rates.  
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25 179 Therefore, it was excluded from the high soil N treatment to ensure sufficient replicates in the  
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27 180 low soil N treatment. For each combination of competition regime and soil N availability  
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29 181 treatment, ten replicates were established. To control for the variation in initial size of the  
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31 182 seedlings, we recorded the initial aboveground size of all tree seedlings (i.e. stem length  
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33 183 measured from root crown, number of leaves and length of largest leaf) before planting. Pots  
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35 184 were watered with tap water every second day to field capacity until the grasses germinated  
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37 185 which occurred within two weeks of planting. Soil N availability treatments therefore  
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39 186 commenced two weeks after planting. Subsequently, once a week 100 ml of nutrient solution  
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41 187 was applied, with low or high N concentrations, for low N: 1  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , 100  $\mu\text{M}$   $\text{KNO}_3$ , 25  
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43 188  $\mu\text{M}$  glutamine and 25  $\mu\text{M}$  arginine; high N: 40  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , 400  $\mu\text{M}$   $\text{KNO}_3$ , 100  $\mu\text{M}$   
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45 189 glutamine and 100  $\mu\text{M}$  arginine, based on soil N availability in previous low vs. high soil N  
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47 190 studies (Simon et al. 2013) based on a low and high N forest site (Dannenmann et al. 2009,  
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49 191 Stoelken et al. 2010, respectively), and on the occurrence of glutamine and arginine in  
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51 192 subtropical Queensland soils (Holst et al. 2012). Other nutrients applied at the same rate in  
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53 193 both N treatments were: 10  $\mu\text{M}$   $\text{AlCl}_3$ , 90  $\mu\text{M}$   $\text{CaCl}_2$ , 7  $\mu\text{M}$   $\text{FeSO}_4$ , 6  $\mu\text{M}$   $\text{K}_2\text{HPO}_4$ , 50  $\mu\text{M}$   
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3 194 KCl, 24  $\mu\text{M}$   $\text{MnCl}_2$ , 20  $\mu\text{M}$  NaCl and 70  $\mu\text{M}$   $\text{MgCl}_2$ . The experiment was conducted in a  
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5 195 naturally lit greenhouse at The University of Queensland, St. Lucia Campus (Brisbane,  
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7 196 Australia), between April and June 2015. Average temperature recorded in the greenhouse  
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9 197 was  $24.7 \pm 3.7^\circ\text{C}$  during the day and  $20.3 \pm 1.8^\circ\text{C}$  at night, reaching a minimum of  $13.5^\circ\text{C}$  and a  
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11 198 maximum of  $35.3^\circ\text{C}$ .

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15 200 *<sup>15</sup>N uptake experiments and harvest*

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18 201 Eight weeks after commencing the N treatments (and ten weeks after planting), the net  
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20 202 inorganic (i.e. ammonium, nitrate) and organic (i.e. glutamine, arginine) N uptake capacity of  
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22 203 fine roots of tree seedlings and grasses was quantified following the <sup>15</sup>N enrichment technique  
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24 204 described by Gessler et al. (1998) as modified by Simon et al. (2010) using fine roots still  
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26 205 attached to the individuals. For this, we carefully removed the plants from the pots and  
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28 206 washed the roots to remove adherent soil particles. Fine roots were then incubated for a 2 h  
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30 207 period in 4 ml artificial nutrient solution containing all four N sources (as described above),  
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32 208 but with one of the sources labelled as either <sup>15</sup>N-ammonium, <sup>15</sup>N-nitrate, <sup>13</sup>C/<sup>15</sup>N-glutamine  
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34 209 or <sup>13</sup>C/<sup>15</sup>N-arginine. Amino acids were <sup>13</sup>C/<sup>15</sup>N double-labelled to determine whether they  
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36 210 were taken up as intact molecules or degraded over the time of incubation (Simon et al. 2011).  
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38 211 Additionally, we used artificial soil solution without <sup>15</sup>N-label to account for the natural  
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40 212 abundance of <sup>15</sup>N in the roots of each species for all treatment levels. Plants were incubated in  
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42 213 either low or high N artificial soil solution matching the soil N availability treatment to which  
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44 214 they had been subjected. Root incubation occurred between 10 am and 2 pm to avoid diurnal  
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46 215 variation in N uptake (Gessler et al. 2002), i.e. the incubation period of 2 h for any given  
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48 216 individual sample started between 10 am and 12 pm and ended between 12 and 2 pm. For the  
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50 217 duration of the <sup>15</sup>N incubation experiments, the remaining seedling's roots were carefully  
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52 218 wrapped in wet tissue to prevent drying out. A total of 6 replicate root samples were set up for  
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54 219 each of the four N sources (plus controls for natural abundance) per species, competition  
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220 regime and soil N availability level. Depending on the size of the root system for an  
221 individual plant, one to four N sources plus control were tested per individual. After the  
222 incubation period, fine roots were carefully cut, washed twice in 0.5 M CaCl<sub>2</sub> solution to  
223 remove the incubation solution from the root surface, and dried with tissue. Following the <sup>15</sup>N  
224 uptake experiments, we separated the plants into above- and belowground organs, i.e. the tree  
225 seedlings into leaves, stem and roots, and the grasses into shoots and roots, since no stem had  
226 developed. We determined the fresh weight (fw) of all plant tissues and the incubated fine  
227 roots, oven dried the samples at 65°C for 72 hours and determined their dry weight (dw) to  
228 obtain biomass indices.

#### 230 *Quantification of total N and C, <sup>15</sup>N and <sup>13</sup>C in fine roots*

231 To quantify <sup>15</sup>N and <sup>13</sup>C enrichment the dried fine roots were ground using a ball mill  
232 (TissueLyser, Retsch, Haan, Germany). Aliquots of 1.2-2.5 mg of the resulting homogeneous  
233 fine powder were weighed into 4x6 mm tin capsules (IVA Analysentechnik,  
234 Meerbusch, Germany), and analysed with an isotope ratio mass spectrometer (Delta V  
235 Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyser (Euro  
236 EA, Eurovector, Milan, Italy).  $\Delta$  values were calculated using a laboratory standard  
237 (acetanilide) that was part of every sequence in intervals, and also used in different weights to  
238 determine isotope linearity of the system. The laboratory standard was calibrated against  
239 different international standards from IAEA (Vienna): for <sup>15</sup>N USGS 40 ( $\delta^{15}\text{N}_{\text{Air}} = -4.5 \text{ ‰}$ ),  
240 IAEA 600 ( $\delta^{15}\text{N}_{\text{Air}} = +1.0 \text{ ‰}$ ), IAEA N2 ( $\delta^{15}\text{N}_{\text{Air}} = +20.3 \text{ ‰}$ ), USGS 41 ( $\delta^{15}\text{N}_{\text{Air}} = +47.6 \text{ ‰}$ ),  
241 USGS 26 ( $\delta^{15}\text{N}_{\text{Air}} = +53.7 \text{ ‰}$ ), and USGS 32 ( $\delta^{15}\text{N}_{\text{Air}} = +180 \text{ ‰}$ ), and for <sup>13</sup>C IAEA 600  
242 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -27.77 \text{ ‰}$ ), IAEA 3 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -24.72 \text{ ‰}$ ), and IAEA CH6 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -10.45$ ).  
243 <sup>15</sup>N results of highly enriched samples were finally corrected with different enriched <sup>15</sup>N  
244 standards (from 0.437 to 0.734 at %) from Fischer Analysen Instrumente (Leipzig, Germany).  
245  $\Delta$  values are defined as  $\delta \text{ [‰]} = (\text{R}_{\text{SA}} / \text{R}_{\text{Std}} - 1) * 1000$ , where R is the ratio of heavy isotope

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3 246 against light isotope (i.e.  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ), SA is sample and Std the international  
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5 247 reference for the respective element (V-PDB for  $^{13}\text{C}$  and air- $\text{N}_2$  for  $^{15}\text{N}$ ). For each of the four  
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7 248 N sources, we then calculated net N uptake capacity ( $\text{nmol N g}^{-1} \text{fw h}^{-1}$ ) based on the  
8  
9 249 incorporation of  $^{15}\text{N}$  into root fresh weight according to Gessler et al. (1998): net N uptake  
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11 250 capacity =  $((^{15}\text{N}_i - ^{15}\text{N}_c) * \text{N}_{\text{tot}} * \text{dw} * 10^5) / (\text{MW} * \text{fw} * t)^{-1}$ , where  $^{15}\text{N}_i$  and  $^{15}\text{N}_c$  are the atom% of  $^{15}\text{N}$   
12  
13 251 in labelled ( $\text{N}_i$ ) and control plants ( $\text{N}_c$ , natural abundance), respectively,  $\text{N}_{\text{tot}}$  is the total N  
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15 252 percentage, MW is the molecular weight ( $^{15}\text{N g mol}^{-1}$ ), and t represents the incubation time  
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17 253 (120 min). Based on  $^{13}\text{C}$  incorporation, net uptake capacity of glutamine and arginine was ~  
18  
19 254 60% and ~ 95% respectively, compared to that based on  $^{15}\text{N}$  incorporation, which indicates  
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21 255 (1) the degradation of amino acids in the solution or on the root surface, and/or (2) the  
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23 256 respiration of amino acid-derived C inside the roots (Simon et al. 2011).  
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### 28 258 *Statistical analysis*

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31 259 For all species, we tested for differences between treatments for total biomass and  
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33 260 root:shoot ratio as well as net uptake capacity of the four N sources by performing  
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35 261 Permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between  
36  
37 262 samples (Anderson et al. 2008). PERMANOVA was chosen as a non-parametric univariate  
38  
39 263 test equivalent to ANOVA (Anderson et al. 2008). We performed two-way PERMANOVAs  
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41 264 using “competition regime” and “soil N availability” as orthogonal factors. For the tree  
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43 265 species, the “competition regime” factor had three levels: control, competition with  
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45 266 *Pennisetum*, and competition with *Cynodon*. Moreover, for the tree seedlings biomass and  
46  
47 267 root:shoot ratio analyses, initial size measurements of the tree seedlings were included as a  
48  
49 268 covariate. For the grass species, the three levels were: control, competition with *Acacia*, and  
50  
51 269 competition with *Podocarpus*. The “soil N availability” factor included two levels: high N  
52  
53 270 and low N for *Acacia*, *Podocarpus* and *Pennisetum*. For the grass *Cynodon*, we performed  
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55 271 one-way PERMANOVAs, considering the factor “competition regime” with three levels:  
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3 272 control, competition with *Acacia*, and competition with *Podocarpus*, all at low soil N  
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5 273 availability as described above. When a significant interaction between factors was found,  
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7 274 *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences in net  
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9 275 N uptake capacity between species, one-way PERMANOVAs were performed on the control  
10  
11 276 data (i.e. no competition), using species as factor, for each N form. To test for preferences in  
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13 277 net uptake capacity of the four N sources for a given species, one-way PERMANOVAs were  
14  
15 278 performed, using N source as factor, for each combination of competition regime and soil N  
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17 279 availability treatment. All analyses were performed using PRIMER 6.0 with the  
18  
19 280 PERMANOVA+ add-on (PRIMER-E Ltd, Plymouth, UK), and figures were prepared in  
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21 281 SigmaPlot 13.0 (Systat Software Inc., San Jose, USA).  
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## 26 283 **Results**

### 28 284 *Effects of competition regime and soil N availability on biomass and root:shoot ratio*

30  
31 285 Native tree seedlings and exotic grasses responded differently in biomass production  
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33 286 depending on competition regime and soil N availability (Figure 1). For the tree species,  
34  
35 287 neither *Acacia* nor *Podocarpus* differed significantly in total biomass between single and  
36  
37 288 competing seedlings regardless of soil N availability (Figure 1, Supplementary Table 1).  
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39 289 Positive correlations were found for *Acacia* between total biomass and the initial leaf number,  
40  
41 290 for *Podocarpus* between total biomass and both initial leaf number and initial stem length  
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43 291 (both *p*-perm < 0.001, data not shown). This indicates that total biomass at the end of the  
44  
45 292 experiment was a function of initial tree seedling size. For the grass *Pennisetum*, total biomass  
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47 293 was significantly reduced in competition with *Acacia* or *Podocarpus* compared to the control  
48  
49 294 at high soil N availability, and it was also significantly reduced at low compared to high soil  
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51 295 N availability irrespective of the competition regime (Figure 1, Supplementary Table 2). For  
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53 296 the grass *Cynodon*, competition with *Acacia* significantly reduced total biomass compared to  
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3 297 competition with *Podocarpus* or control (at low soil N availability) (Figure 1, Supplementary  
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5 298 Table 2).

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9 300 Root:shoot ratio was not affected by soil N availability or competition regime for both  
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11 301 tree species as well as competition regimes for the grass *Cynodon* at low soil N availability  
12  
13 302 (Figure 1). For the grass *Pennisetum*, root:shoot ratio was significantly lower in competition  
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15 303 with *Podocarpus* compared to competition with *Acacia* or controls irrespective of soil N  
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17 304 availability (Figure 1).

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22 306 *Effects of competition regime and soil N availability on inorganic and organic net N uptake*  
23  
24 307 *capacity*

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26 308 Inorganic and organic net N uptake capacity of all species increased significantly with  
27  
28 309 high compared to low soil N availability, whereas the responses to competition regime  
29  
30 310 differed depending on the species (Figures 2 and 3, Supplementary Table 3). Inorganic and  
31  
32 311 organic net N uptake capacity of *Acacia* seedlings increased with higher soil N availability  
33  
34 312 regardless of competition regime. At high soil N availability, net ammonium uptake capacity  
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36 313 of *Acacia* seedlings competing with the grass *Pennisetum* increased significantly compared to  
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38 314 seedlings growing without competition (Figure 2, Supplementary Table 4), but no change for  
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40 315 nitrate (Figure 2), glutamine-N or arginine-N (Figure 3) was found at either low or high soil N  
41  
42 316 availability. Similarly, inorganic N (Figure 2) and arginine-N (Figure 3) net uptake capacity  
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44 317 of *Podocarpus* increased with high compared to low soil N availability irrespective of  
45  
46 318 competition regime. However, net glutamine-N uptake capacity increased with higher soil N  
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48 319 availability only for *Podocarpus* seedlings grown in competition with *Pennisetum* (Figure 3,  
49  
50 320 Supplementary Table 4). At high soil N availability, competition with *Pennisetum* increased  
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52 321 net glutamine-N uptake capacity of *Podocarpus* seedlings compared to seedlings growing  
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54 322 alone (Figure 3); however, net ammonium, nitrate (Figure 2), and arginine-N (Figure 3)

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3 323 uptake capacity were not affected. For the grass *Pennisetum*, net ammonium and organic N,  
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5 324 but not nitrate uptake capacity were significantly higher at high compared to low soil N  
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7 325 availability (Figures 2 and 3). For *Pennisetum*, competition regime had no significant effect  
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9 326 on organic (Figure 3) and inorganic (Figure 2) net N uptake capacity at either low or high soil  
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11 327 N availability. At low soil N availability, nitrate, but not ammonium or organic net N uptake  
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13 328 capacity of the grass *Cynodon* was significantly higher when grown in competition with  
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15 329 *Podocarpus* compared to that with *Acacia*, or control (Figures 2 and 3, Supplementary Table  
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17 330 4).

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### 21 22 332 *Differences between species with regard to inorganic and organic N acquisition*

23  
24 333 In the absence of interspecific competition (i.e. single grown tree seedlings or grasses  
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26 334 growing in monocultures), species differed in their inorganic and organic N acquisition  
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28 335 strategies depending on the N source: seedlings of *Podocarpus* took up significantly less  
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30 336 nitrate than seedlings of *Acacia* and the grass *Pennisetum*, irrespective of soil N availability  
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32 337 (Table 1). However, glutamine-N acquisition of *Pennisetum* was significantly lower than that  
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34 338 of *Podocarpus*, which in turn was lower than that of *Acacia* seedlings at both low and high  
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36 339 soil N availability (Table 1). No differences were found between any of the species with  
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38 340 regard to ammonium and arginine-N acquisition (Table 1).

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43 342 General preference patterns of the different N forms were found for each species  
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45 343 within each competition regime depending on soil N availability: At low soil N availability,  
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47 344 both tree species preferred nitrate and glutamine-N over ammonium and arginine-N  
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49 345 (Supplementary Table 5). However, at high soil N availability, this pattern was reversed with  
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51 346 ammonium and arginine-N being favoured over nitrate and glutamine-N for both tree species  
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53 347 (Table 2, Supplementary Table 5). For the grasses, inorganic N sources, particularly nitrate,  
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3 348 were preferred over organic N sources (regardless of soil N availability for *Pennisetum*)  
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5 349 (Table 2, Supplementary Table 6).  
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9 351 **Discussion**

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11 352 Our study provides novel insight into the species-specific growth and N acquisition  
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13 353 patterns in native tree seedlings and exotic grasses competing for N. Depending on soil N  
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15 354 availability, native tree seedlings responded to competition with increases in N acquisition  
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17 355 only, whereas the responses of the grasses in terms of biomass and N acquisition varied with  
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19 356 species and soil N availability. These results highlight that quantifying underlying  
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21 357 mechanisms complements biomass indices to advance the understanding of interspecific  
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23 358 competition (Trinder et al. 2013). Furthermore, the lack of response with regard to biomass in  
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25 359 the tree seedlings compared to the grasses suggests that N storage capacity plays a role in the  
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27 360 growth response as well.  
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33 362 *Native tree seedlings increase N acquisition in competition with exotic grasses at high soil N*  
34  
35 363 *availability*

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37 364 At high soil N availability, both tree species responded similarly to competition with  
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39 365 the exotic grass *Pennisetum* by increasing N acquisition. However, growth and biomass  
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41 366 allocation were unaffected, which indicates that they might allocate the newly acquired N to  
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43 367 storage rather than investing into growth, thereby reducing N availability for their competitors  
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45 368 (Millard and Grelet 2010). These similarities between *Acacia* and *Podocarpus* in terms of net  
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47 369 N uptake capacity and growth responses to competition with grasses indicate that both tree  
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49 370 species use a common strategy, which is rather unexpected given the different successional  
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51 371 status, growth strategies and physiological characteristics of the two tree species. *Acacia* is an  
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53 372 early successional, fast-growing and N<sub>2</sub> fixing species, while *Podocarpus* is a slow-growing,  
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55 373 late successional species (Smith et al. 2008). Thus, since fast-growing species would require  
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3 374 higher amounts of N to meet their growth rate (Poorter et al. 2012), the observed increase in  
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5 375 net N uptake capacity when growing in competition would be expected in *Acacia* and not in  
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7 376 *Podocarpus*. However, previous studies have shown that *Acacia* seedlings are capable of N-  
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9 377 fixation (Sun et al. 1992), and that root nodulation in *Acacia* species is stimulated by  
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11 378 competition with grasses (Cramer et al. 2007). Therefore the increased requirements of  
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13 379 *Acacia* due to competition would probably be mitigated by its ability to fix atmospheric N<sub>2</sub>  
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15 380 (not quantified in our study) and thus reduce to an extent its dependency on external N supply.  
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17 381 Moreover, the lack of response of *Acacia* to competition in terms of N uptake at low soil N  
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19 382 availability in our study might also be attributed to a greater reliance on N<sub>2</sub> fixation under  
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21 383 limiting N conditions (Pfautsch et al. 2009).  
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28 385 Tree species differed in the acquired N forms: in competition fast-growing *Acacia*  
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30 386 acquired more ammonium compared to single grown individuals, while slow-growing  
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32 387 *Podocarpus* used glutamine-N. This preference of inorganic vs. organic N forms when  
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34 388 competing might reflect different N acquisition strategies between fast- and slow-growers as  
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36 389 reported for temperate tree seedlings (Simon et al. 2010, Simon et al. 2014, Li et al. 2015).  
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38 390 The similar responses found here might be an early indication that, across biomes, N  
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40 391 acquisition of trees is linked to growth strategy. These increases in net N uptake capacity as a  
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42 392 response to competition were found only at high soil N availability suggesting mediation of N  
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44 393 acquisition by soil N availability (e.g. Simon et al. 2013, Li et al. 2015). For *Acacia*, this  
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46 394 could be related to a reduced nodule activity at high soil N concentrations, as it has been  
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48 395 suggested that N acquisition from the soil, rather than N<sub>2</sub> fixation, is energetically more  
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50 396 efficient for this species when inorganic N is readily available (Pfautsch et al. 2009). With  
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52 397 regard to *Podocarpus*, an increase in organic N acquisition with competition was also  
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54 398 reported for a temperate slow-growing tree species at high, but not low soil N (Li et al. 2015).  
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3 399 Our results constitute a further example of increased N acquisition under competition with  
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5 400 high N availability, but in a subtropical environment. Particularly in the view of increased  
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7 401 atmospheric N deposition in the future (Rennenberg and Dannenmann 2015, Jia et al. 2016),  
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9 402 the outcomes of competition in currently N-limited habitats are likely to change.

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14 404 At low soil N availability, competition with grasses did not result in changes in N  
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16 405 acquisition or biomass production and allocation for both tree species. Similarly, previous  
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18 406 studies have reported a lack of effects of competition with the grass *Cynodon dactylon* on the  
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20 407 growth (i.e. biomass, basal diameter and stem height) of a different *Acacia* species (*A. smallii*  
21  
22 408 Isely) on non-supplemented native soil (Cohn et al. 1989). Nevertheless, this grass species  
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24 409 hinders the growth of native tree seedlings in the field and is reported as a threat to restoration  
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26 410 efforts in subtropical Australia (M. Amos, pers. comm.). Since we did not observe these  
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28 411 negative effects in our study, it is likely that the impacts of the competition with *Cynodon* on  
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30 412 tree seedling growth are evident at later developmental stages of both the grass and the tree  
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32 413 species than the ones considered here. The lack of response to competition of the tree  
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34 414 seedlings regarding total biomass could also be due to the short duration of our experiment,  
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36 415 considering the relatively slow growth of tree seedlings compared to grasses. Previous studies  
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38 416 reporting negative effects of grasses on *Acacia* measured responses over longer periods, e.g. 3  
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40 417 years (Messina and Barton 1985), and 8 years (Florentine and Westbrooke 2004).  
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42 418 Furthermore, the effect of the grass on tree seedlings may be mediated by soil N availability:  
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44 419 in our study, the effects of competition with *Cynodon* were not investigated at high soil N  
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46 420 availability, but it has been reported that competition with *Cynodon* combined with an  
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48 421 increase in soil nutrient availability results in 70-90% reduction in growth of *A. smallii* (Cohn  
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50 422 et al. 1989).

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3 424 *Exotic grasses respond to competition for N depending on the competitor and soil N*  
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5 425 *availability*  
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7 426 For the exotic grasses, four strategies were identified which depended on the  
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9 427 competing species and soil N availability: (1) *Pennisetum* biomass was reduced in  
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11 428 competition with both tree species compared to the control at high, but not low soil N  
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13 429 availability suggesting that the outcome of competitive interactions between native tree  
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15 430 seedlings and *Pennisetum* depends on soil N availability. Moreover, the biomass of  
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17 431 *Pennisetum* at high soil N availability, even if reduced by competition with the tree seedlings,  
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19 432 was still higher than its biomass at low soil N in the absence of interspecific competition,  
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21 433 suggesting that, at least in the short-term, the grass is more successful than the tree seedlings  
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23 434 at high soil N availability, despite the observed negative effects of competition (2) The grass  
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25 435 *Cynodon* showed a similar strategy as *Pennisetum*, i.e. a reduction in biomass but no change  
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27 436 in N acquisition, but only when competing with *Acacia*, but not *Podocarpus*, indicating that  
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29 437 for both grass species the response to competition depends on the competitor. (3) Resource  
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31 438 allocation shifted to aboveground at the expense of belowground tissue production (Aerts et  
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33 439 al. 1991) for *Pennisetum* competing with *Podocarpus* at low and high soil N. This suggests  
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35 440 that, despite the fact that shading from the tree seedlings was not apparent, the potential  
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37 441 competition for light is more relevant for *Pennisetum* than competition for belowground  
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39 442 resources (Poorter et al. 2012), thus not leading to an increase in N acquisition. However,  
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41 443 these results were not observed when grown in competition with *Acacia* or for the grass  
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43 444 *Cynodon* indicating that this biomass allocation strategy of *Pennisetum* also depends on the  
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45 445 competing species, but not soil N availability. (4) Competition with *Podocarpus*, but not  
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47 446 *Acacia*, resulted in increased net nitrate uptake in *Cynodon*, but not *Pennisetum* suggesting  
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49 447 different N acquisition strategies between the two exotic grass species that vary with the  
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51 448 competing tree species. Overall, our results suggest that the outcome of competitive  
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3 449 interactions between native tree seedlings and exotic grasses are species-specific and  
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5 450 mediated by soil N availability.

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9 452 *Differences in N acquisition strategies between species suggest potential for niche*  
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11 453 *differentiation*

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13 454 All four species took up both inorganic and organic N forms, although specifics varied  
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15 455 according to N forms. Nitrate acquisition was higher in *Pennisetum* and *Acacia* compared to  
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17 456 *Podocarpus*, consistent with their successional status and the low abundance of nitrate in late-  
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19 457 successional forest soils as well as a reduced nitrate use capacity in late-successional tree  
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21 458 species (Kronzucker et al. 1997, Britto and Kronzucker 2013). At sites with higher soil nitrate  
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23 459 availability, e.g. disturbed areas, the grass *Pennisetum* would have a competitive advantage  
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25 460 over *Podocarpus*, but not *Acacia*, highlighting the importance of species-specific  
26  
27 461 consideration for reforestation programs. Moreover, glutamine-N acquisition was higher for  
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29 462 *Acacia* than *Podocarpus*, contrary to what we expected according to their successional status,  
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31 463 since soil amino acid content generally increases as succession progresses (Werdin-Pfisterer  
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33 464 et al. 2009). Nevertheless, glutamine-N acquisition was higher in both tree species compared  
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35 465 to *Pennisetum* indicating a potential for avoidance of competition via the use of the different  
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37 466 N forms (Simon et al. 2017). Overall, that plants take up organic N forms is now well  
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39 467 established and has been reported for numerous plant species and ecosystems (Näsholm et al.  
40  
41 468 2009). Moreover, its ecological relevance is increasingly recognised due to the resulting niche  
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43 469 differentiation and consequences in terms of species coexistence (Näsholm et al. 2009, Simon  
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45 470 et al. 2014, Li et al. 2015, Andersen et al. 2017, Uscola et al. 2017).

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49 472 Comparing within tree and grass species, higher soil N availability resulted in  
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51 473 increased inorganic and organic N acquisition in general, which for *Pennisetum* was  
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53 474 combined with increased biomass production (for *Cynodon* data is only available for low soil  
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3 475 N). This increase in grass biomass production with increasing soil N availability while there  
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5 476 was no short-term response of the tree seedlings suggests a potential advantage for the grass  
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7 477 in habitats on N-rich soils or in future scenarios with increased atmospheric N deposition  
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9 478 compared to the native trees. This link between high resource availability and success of  
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11 479 invasive species has been explored in previous studies (e.g. Badgery et al. 2005, Gao et al.  
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13 480 2014, Yelenik et al. 2017), and has been linked to invader traits related to exploitation and  
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15 481 efficient use of resources, which allows for better growth (Huangfu et al. 2016, Yelenik et al.  
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17 482 2017). In our study, although competition with seedlings of both tree species reduced the total  
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19 483 biomass of *Pennisetum* compared to *Pennisetum* grown in controls, the increase in  
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21 484 *Pennisetum* biomass in response to higher soil N availability was of greater magnitude.  
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23 485 Increases in the productivity of *Pennisetum* with N fertilization resulting in its dominance at  
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25 486 fertilised sites have been reported before (Mears 1970, Barton et al. 2008), and under such  
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27 487 conditions, *Pennisetum* might be more competitive than tree seedlings. Moreover, the  
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29 488 potential of *Acacia* to induce increases in soil N availability over time as a consequence of its  
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31 489 N<sub>2</sub> fixing ability has been shown before in silvopastoral systems (Power et al. 2003). Thus,  
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33 490 increased N supply as a consequence of the use of N<sub>2</sub> fixing tree species such as *Acacia* might  
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35 491 favour the growth and potential dominance of *Pennisetum* at restoration sites.  
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#### 41 493 *Methodological considerations*

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44 494 Our experimental approach has several limitations. First, the competitor grasses were  
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46 495 grown from seeds, whereas the trees were purchased as seedlings. This set up was selected  
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48 496 because our aim was to mimic the actual timing in a restoration setting under field conditions  
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50 497 for which tree species are planted as seedlings for reforestation and grasses arrive at a later  
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52 498 time. Thus, a comparison between trees and grasses cannot exclude differences due to  
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54 499 variation in developmental stages between tree species and grasses. Specifically, the lack of  
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56 500 response in seedling biomass reported here might not persist with older or larger grasses.  
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3 501 Second, tree seedlings differed in their age at the start of the experiment which might have  
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5 502 influenced their responses to competition and/or soil N availability in our experiment.  
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7 503 However, given the differences in growth rates between the tree species used in this study,  
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9 504 seedlings of similar age would have likely differed in their initial above- and belowground  
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11 505 dimensions, thereby introducing another variable. Third, the experiment ran for eight weeks  
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13 506 which might not be sufficient time for a response in tree seedling biomass, however they did  
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15 507 respond with regard to N acquisition from the soil. Finally, the artificial N solution used in  
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17 508 this study is based on a low and high N forest site (Dannenmann et al. 2009, Stoelken et al.  
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19 509 2010, respectively) as well as the occurrence of glutamine and arginine in soils of subtropical  
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21 510 Queensland (Holst et al. 2012), but not actual measured concentrations at subtropical  
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23 511 restoration sites.  
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### 28 513 *Conclusions*

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31 514 In our study, native tree seedlings and exotic grasses responded to competition with  
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33 515 two distinct response types: (1) a reduction in biomass, but no change in N acquisition, (2) an  
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35 516 increase in N acquisition without a change in biomass. This outcome depended on the  
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37 517 competing species and soil N availability. Thus, we suggest that these aspects be considered  
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39 518 also for ecosystem restoration and management, with special attention to the potential  
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41 519 feedbacks between native trees possessing traits such as N<sub>2</sub> fixing ability and exotic grasses  
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43 520 that may benefit from the resulting increase in soil N availability. Exotic grasses such as  
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45 521 *Pennisetum* and *Cynodon* are considered a high priority for control in Australia and their  
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47 522 suppression can promote the reestablishment of woody vegetation, thus determining the  
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49 523 success of subtropical rainforest restoration programs (Florentine and Westbrooke 2004,  
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51 524 Downey et al. 2010, Shoo and Catterall 2013). We found no common N acquisition strategy  
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53 525 across species in response to competition between native trees and invasive grasses indicating  
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55 526 the need to consider species-specific approaches in restoration management plans.  
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3 776 **Figure legends**

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5 777 Figure 1. Total biomass and root:shoot ratio of *Acacia melanoxylon*, *Podocarpus elatus*,  
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7 778 *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability under  
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9 779 different competition regimes. For *C. dactylon*, only low soil N data is available. comp  
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11 780 *Pennisetum* = competition with *P. clandestinum*; comp *Cynodon* = competition with *C.*  
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13 781 *dactylon*; comp *Acacia* = competition with *A. melanoxylon*; comp *Podocarpus* = competition  
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15 782 with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters  
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17 783 indicate significant differences between competition regimes within a specific soil N  
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19 784 availability treatment, and asterisks indicate significant differences between soil N availability  
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21 785 treatments, detected using permutational analysis of variance ( $p < 0.05$ ).  
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26 787 Figure 2. Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) net uptake capacity ( $\text{nmol N g}^{-1} \text{fw h}^{-1}$ ) by  
27  
28 788 fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon*  
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30 789 *dactylon* at low and high soil N availability under different competition regimes. For *C.*  
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32 790 *dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P.*  
33  
34 791 *clandestinum*; comp *Cynodon* = competition with *C. dactylon*; comp *Acacia* = competition  
35  
36 792 with *A. melanoxylon*; comp *Podocarpus* = competition with *P. elatus*. Box plots show mean  
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38 793 (dotted line) and median (straight line). Different letters indicate significant differences  
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40 794 between competition regimes within a specific soil N availability treatment, and asterisks  
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42 795 indicate significant differences between soil N availability treatments detected using  
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44 796 permutational analysis of variance ( $p < 0.05$ ).  
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50 798 Figure 3. Glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity ( $\text{nmol N g}^{-1} \text{fw h}^{-1}$ )  
51  
52 799 by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and  
53  
54 800 *Cynodon dactylon* at low and high soil N availability under different competition regimes. For  
55  
56 801 *C. dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P.*  
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3 802 *clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition  
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5 803 with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean  
6  
7 804 (dotted line) and median (straight line). Different letters indicate significant differences  
8  
9 805 between competition regimes within a specific soil N availability treatment, and asterisks  
10  
11 806 indicate significant differences between soil N availability treatments detected using  
12  
13 807 permutational analysis of variance ( $p < 0.05$ ).  
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For Peer Review

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3 1 **Responses to competition for nitrogen between subtropical native tree**  
4  
5 2 **seedlings and exotic grasses are species-specific and mediated by soil N**  
6  
7 **availability**  
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11 4  
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37  
38 17 **Keywords:** ammonium, interspecific competition, net nitrogen uptake capacity, nitrogen  
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40 acquisition, nitrate, organic nitrogen, reforestation, subtropics  
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45 20 **Running head:** Soil N mediates tree-grass competition  
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1  
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3 21 **Abstract**

4  
5 22 Competitive interactions between native tree seedlings and exotic grasses frequently  
6  
7 23 hinder forest restoration. We investigated the consequences of competition with exotic grasses  
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9 24 on the growth and net nitrogen (N) uptake capacity of native rainforest seedlings used for  
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11 25 reforestation depending on soil N availability and N source. Tree seedlings and grasses were  
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13 26 grown in the greenhouse in different competition regimes (one tree species vs. one grass  
14  
15 27 species) and controls (grass monocultures or single tree seedlings) at low and high soil N.  
16  
17 28 After eight weeks, we quantified net N uptake capacity using <sup>15</sup>N-labelled organic (i.e.  
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19 29 glutamine and arginine) and inorganic (i.e. ammonium and nitrate) N sources and biomass  
20  
21 30 indices. Depending on soil N availability, we observed different species-specific responses to  
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23 31 growth and N acquisition. Tree seedlings generally increased their net N uptake capacity in  
24  
25 32 response to competition with grasses, although overall seedling growth was unaffected. In  
26  
27 33 contrast, the responses to competition by the grasses were species-specific and varied with  
28  
29 34 soil N availability. The different N acquisition strategies suggest the avoidance of competition  
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31 35 for N between trees and grasses. Overall, the results highlight that quantifying underlying  
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33 36 mechanisms of N acquisition complements the information on biomass allocation as a  
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35 37 measure of responses to competition, particularly with varying environmental conditions.  
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## 38 Introduction

39 Plant-plant interactions shape the structure of plant communities (Callaway and  
40 Walker 1997, Brooker 2006, Tylianakis et al. 2008). Their magnitude and direction are  
41 influenced by factors including life stage, physiological requirements as well as  
42 environmental conditions (Callaway and Walker 1997). The interplay between these biotic  
43 and abiotic factors determines the outcome of species interactions which in turn affects the  
44 coexistence and spatial distribution of species (Brooker et al. 2008, Soliveres and Maestre  
45 2014). A main interaction is the competition for resources such as soil nutrients. The ability of  
46 plants to acquire nutrients is determined by morphological and physiological traits, such as  
47 uptake rates of nutrients (Casper and Jackson 1997). By modifying nutrient uptake in  
48 response to environmental cues, such as nutrient~~their~~ availability, plants can be more  
49 competitive but the extent of such response varies (Hodge 2004).

50  
51 Competitive interactions between native and exotic plant species are relevant in the  
52 context of global environmental change, because there is evidence that the predicted changes  
53 in the abiotic environment will likely influence competition in favor of exotic species to  
54 (Bradley et al. 2010, Verlinden et al. 2014). The superior competitive ability of exotic plant  
55 species is frequently a key factor promoting invasions, with exotic species able to better  
56 exploit limited resources, to the detriment of native species (D'Antonio and Vitousek 1992,  
57 Gioria and Osborne 2014). Changing nutrient status, such as increased nitrogen (N)  
58 availability with atmospheric deposition, can alter plant growth and biomass allocation in a  
59 species-specific manner, and consequently results in shifts in the outcome of interspecific  
60 competition (Rennenberg et al. 2009). Here, we investigate the competition between exotic  
61 and native plants comparing the response strategies of different species, and how resource  
62 availability modulates these interactions. A focus on N physiology is warranted because N is  
63 quantitatively the most important of soil-acquired nutrients (Marshner 1995), limits plant

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2  
3 64 growth in most ecosystems (Agren et al. 2012), drives competition (Eller and Oliveira 2017),  
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5 65 and N availability and uptake can vary considerably in response to environmental change  
6  
7 66 (BassiriRad 2000, Kuster et al. 2016). Since most studies focus on biomass production as  
8  
9 67 indicator of the magnitude and direction of competitive interactions, even though other factors  
10  
11 68 besides competition may influence biomass, (Trinder et al. 2013), we also examined net N  
12  
13 69 uptake as a process that underlies the interaction between native and exotic species, because  
14  
15 70 exotic species might alter soil N availability, show strong preferences to certain N forms  
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17 71 and/or interfere with the N acquisition of native species, therefore affecting their performance  
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19  
20 72 (Fraterrigo et al. 2011, Huangfu et al. 2016, Eller and Oliveira 2017).  
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24 74 While earlier studies have focused mainly on the acquisition of inorganic N, organic N  
25  
26 75 uptake has received increasing attention due to its potential to drive niche differentiation and  
27  
28 76 species coexistence, and therefore ecosystem stability (Schmidt and Stewart 1999, McKane et  
29  
30 77 al. 2002, Kielland et al. 2006, Näsholm et al. 2009, Ashton et al. 2010). A plant's ability to  
31  
32 78 use a wider array of N sources can result in competition avoidance (e.g. Miller et al. 2007,  
33  
34 79 Simon et al. 2010, Simon et al. 2014, Li et al. 2015). Studies that have quantified the uptake  
35  
36 80 of organic and inorganic N forms found species-specific preferences for different N forms  
37  
38 81 (e.g. Persson et al. 2003, Weigelt et al. 2005, Simon et al. 2010, Li et al. 2015). For example,  
39  
40 82 European beech (*Fagus sylvatica*) shows a preference for organic rather than inorganic N,  
41  
42 83 whereas the opposite is true for sycamore maple (*Acer pseudoplatanus*) (Li et al. 2015). In a  
43  
44 84 study on excised roots of native and exotic grasses of Australian savannas, a general  
45  
46 85 preference for ammonium was found, but preferences for nitrate and the amino acid glycine  
47  
48 86 were species-specific (Rossiter-Rachor et al. 2009). Furthermore, the uptake capacity of  
49  
50 87 different N sources is influenced by interspecific competition (e.g. Miller et al. 2007, Simon  
51  
52 88 et al. 2010, Simon et al. 2014, Li et al. 2015). For example, when grown in competition with  
53  
54 89 sycamore maple, the capacity for organic N uptake of European beech seedlings was reduced  
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3 90 (Simon et al. 2014). However, the responses of competition with regard to N acquisition are  
4  
5 91 also influenced by environmental factors (Simon et al. 2014, Li et al. 2015). Overall, most  
6  
7 92 studies that have examined the acquisition of different N forms in context of competition have  
8  
9 93 focused either on herbaceous or woody species in temperate ecosystems, rather than the  
10  
11 94 interactions between trees and grasses. These, however, are important in the context of  
12  
13 95 conservation and ecosystem restoration, because the successful establishment of tree seedlings  
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15 96 (commonly planted in order to restore a plant community) often depends on the outcome of  
16  
17 97 their interaction with grasses (Erskine et al. 2005, Doust et al. 2008, Elgar et al. 2014), where  
18  
19 98 N is likely to play a determinant role (Coll et al. 2004, Barbosa et al. 2014).  
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24 100 Tree-grass interactions have been broadly studied in savanna ecosystems measuring  
25  
26 101 the influence of abiotic factors (e.g. light, water and nutrient availability) that mediate the  
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28 102 interaction (e.g. Ludwig et al. 2001, van der Waal et al. 2009, Moustakas et al. 2013) and in  
29  
30 103 studies investigating the long-term coexistence of these contrasting life forms (e.g. Jeltsch et  
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32 104 al. 2000, Sankaran et al. 2004). With regard to forest regeneration, tree-grass interactions have  
33  
34 105 been studied in the context of secondary succession, tree regeneration in forest gaps after  
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36 106 disturbances, or meadows (e.g. Coll et al. 2004, Bloor et al. 2008a, Elgar et al. 2014), where  
37  
38 107 tree seedlings interact with the already established plant community commonly composed of  
39  
40 108 early successional life forms, including grasses. However, research on the effect of  
41  
42 109 herbaceous vegetation on tree seedlings mainly focusses on seedling survival and growth (e.g.  
43  
44 110 Davis et al. 1998, Bloor et al. 2008b, Meli and Dirzo 2013), physiological aspects such as  
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46 111 water relations (e.g. Quinteros et al. 2017) or photosynthesis (e.g. Davis et al. 1999, Loik and  
47  
48 112 Holl 2001), while competition for nutrients, specifically N, has received little attention  
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50 113 despite the potential of belowground competition with grasses to influence tree growth and  
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52 114 establishment (e.g. Cramer et al. 2010) and the influence of soil N availability on the  
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56 115 competition outcome (e.g. Cramer et al. 2012).  
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117 Here, we studied the interactions between native tree seedlings and exotic grasses  
118 because ecological forest restoration efforts are frequently hindered by competition from  
119 exotic grasses (Kanowski et al. 2003, Erskine et al. 2005). In reforestation programs, grasses  
120 successfully establish even after the planting of seedlings without regular weeding. To mimic  
121 these conditions, we used nursery-grown tree seedlings and grasses grown from seed in our  
122 study. We ~~selected~~ ~~these~~ tree species that are relevant in the context of restoration of riparian  
123 zones in subtropical Australia (Catteral and Harrison 2006, Gageler et al. 2014), including an  
124 N<sub>2</sub> fixer. Tree species with the capacity to fix atmospheric N<sub>2</sub> via symbiotic rhizobacteria in  
125 root nodules are commonly used in restoration programs with the intent to improve the overall  
126 N supply at a site (Erskine et al. 2005). Their N<sub>2</sub> fixing ability would make them largely  
127 independent from external soil N supply (Messina and Barton 1985) and potentially alleviate  
128 competition for N (Epron et al. 2006). We conducted a greenhouse experiment to advance the  
129 understanding of whether competition for N plays a role in the interaction between seedlings  
130 of two native tree species and two exotic grass species, and the potential effects of soil N  
131 availability. Our objectives were to (i) determine the effect of interspecific competition on  
132 inorganic and organic N acquisition and biomass indices as response strategies of tree  
133 seedlings and grasses, and (ii) evaluate the impact of low *versus* high soil N availability on the  
134 competition outcome in terms of N acquisition. We hypothesized that tree seedlings and  
135 grasses potentially avoid competition by using different N forms (i.e., fast-growing grasses  
136 using inorganic forms and relatively slow-growing trees using organic forms), that growth and  
137 net N uptake capacity of tree seedlings is negatively affected in the presence of competing  
138 exotic grasses, and that this negative effect is lessened at high soil N availability.

139

## 140 **Materials and Methods**

### 141 *Study species and plant material*

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3 142 We selected two native tree species used in riparian reforestation in the Maleny region  
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5 143 (26.7° S, 152.8° E, Queensland, Australia), angiosperm *Acacia melanoxylon* R.Br (Fabaceae)  
6  
7 144 and gymnosperm *Podocarpus elatus* R.Br. ex Endl. (Podocarpaceae). *A. melanoxylon* is an  
8  
9 145 early successional, N<sub>2</sub> fixing species native to south-east Australia (Smith et al. 2008,  
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11 146 Birnbaum et al. 2012) that occurs in rainforests and wet sclerophyll forests (Doran and  
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13 147 Turnbull 1997). *P. elatus* is a late-successional endemic species that grows in subtropical  
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15 148 rainforests (Smith et al. 2008). Seedlings of *A. melanoxylon* (5 months old, ~ 40 cm tall) and  
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17 149 *P. elatus* (10 months old, ~ 30 cm tall) were obtained from a local nursery (Brush Turkey  
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19 150 Enterprises, Maleny, Australia). We selected tree species with contrasting successional status  
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21 151 to explore potential differences in their N acquisition strategies related to growth rate (Li et al.  
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23 152 2015), and species adaptation to N forms available in soils of different successional status  
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25 153 (e.g. higher nitrate availability in early successional sites compared to late sites) ([Kronzucker](#)  
26  
27 154 [et al. 1997](#), [Britto and Kronzucker 2013](#)). The exotic grasses *Pennisetum clandestinum*  
28  
29 155 Hochst. ex Chiov. (Poaceae) and *Cynodon dactylon* (L.) Pers. (Poaceae) are perennial,  
30  
31 156 originate from Africa and have been introduced to tropical areas around the world (Judd  
32  
33 157 1979). Spreading via rhizomes, stolons and seeds, these grasses naturally occur in grasslands  
34  
35 158 and are common in pastures and lawns in their introduced range (Judd 1979). In subtropical  
36  
37 159 Australia, these two grass species are reported as a threat to restoration efforts, and their  
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39 160 negative effects on the growth of native tree seedlings have been observed in the field (M.  
40  
41 161 Amos, pers. comm.). Grass seeds were purchased from Royston Petrie Seeds (Mudgee,  
42  
43 162 Australia). From here on, species will be referred to by their genus, i.e. *Acacia*, *Podocarpus*,  
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45 163 *Pennisetum* and *Cynodon*.  
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#### 165 *Experimental setup*

54  
55 166 Tree seedlings were planted and grasses were sown from seed into 1 L pots (125 mm  
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57 167 diameter, Anova Solutions, Brisbane, Australia) with a 1:1 mixture of grade A pit sand (grain  
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3 168 size: 0.5 mm) and G2 vermiculite (grain size: 1-2 mm) in interspecific competition or under  
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5 169 control conditions at low and high soil N availability. Competition treatments consisted of one  
6  
7 170 tree individual surrounded by 10-12 individual grasses that were uniformly distributed ~4 cm  
8  
9 171 from the seedling stem. The four species combinations were *Acacia* vs. *Pennisetum*, *Acacia*  
10  
11 172 vs. *Cynodon*, *Podocarpus* vs. *Pennisetum* and *Podocarpus* vs. *Cynodon*. Control treatments  
12  
13 173 consisted either of a single tree seedling or 12 individuals of one grass species (i.e.  
14  
15 174 monoculture). Tree seedlings were not large enough to result in shading for the grasses (A.  
16  
17 175 Bueno, *pers. obs.*). Because competition for N is highest under conditions of N limitation  
18  
19 176 (Wilson and Tilman 1991, Casper and Jackson 1997), all competition regimes received the  
20  
21 177 low soil N treatment. To ensure ~~sufficient~~enough replication, the high soil N treatment was  
22  
23 178 only applied for tree species vs. *Pennisetum* because *Cynodon* had poor germination rates.  
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25  
26 179 Therefore, it was excluded from the high soil N treatment to ensure ~~sufficient~~enough  
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28 180 replicates in the low soil N treatment. For each combination of competition regime and soil N  
29  
30 181 availability treatment, ten replicates were established. To control for the variation in initial  
31  
32 182 size of the seedlings, we recorded the initial aboveground size of all tree seedlings (i.e. stem  
33  
34 183 length measured from root crown, number of leaves and length of largest leaf) before  
35  
36 184 planting. Pots were watered with tap water every second day to field capacity until the grasses  
37  
38 185 germinated which occurred within two weeks of planting. Soil N availability treatments  
39  
40 186 therefore commenced two weeks after planting. Subsequently, once a week 100 ml of nutrient  
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42 187 solution was applied, with low or high N concentrations, for low N: 1  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , 100  $\mu\text{M}$   
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44 188  $\text{KNO}_3$ , 25  $\mu\text{M}$  glutamine and 25  $\mu\text{M}$  arginine; high N: 40  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , 400  $\mu\text{M}$   $\text{KNO}_3$ , 100  
45  
46 189  $\mu\text{M}$  glutamine and 100  $\mu\text{M}$  arginine, based on soil N availability in previous low vs. high soil  
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48  
49 190 N studies (Simon et al. 2013) ~~based on a low and high N forest site (Dannenmann et al. 2009,~~  
50  
51 191 ~~Stoelken et al. 2010, respectively)~~, and on the occurrence of glutamine and arginine in  
52  
53 192 subtropical Queensland soils (Holst et al. 2012). Other nutrients applied at the same rate in  
54  
55 193 both N treatments were: 10  $\mu\text{M}$   $\text{AlCl}_3$ , 90  $\mu\text{M}$   $\text{CaCl}_2$ , 7  $\mu\text{M}$   $\text{FeSO}_4$ , 6  $\mu\text{M}$   $\text{K}_2\text{HPO}_4$ , 50  $\mu\text{M}$

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3 194 KCl, 24  $\mu\text{M}$   $\text{MnCl}_2$ , 20  $\mu\text{M}$  NaCl and 70  $\mu\text{M}$   $\text{MgCl}_2$ . The experiment was conducted in a  
4  
5 195 naturally lit greenhouse at The University of Queensland, St. Lucia Campus (Brisbane,  
6  
7 196 Australia), between April and June 2015. Average temperature recorded in the greenhouse  
8  
9 197 was  $24.7 \pm 3.7^\circ\text{C}$  during the day and  $20.3 \pm 1.8^\circ\text{C}$  at night, reaching a minimum of  $13.5^\circ\text{C}$  and a  
10  
11 198 maximum of  $35.3^\circ\text{C}$ .

13  
14 199

15  
16 200 *<sup>15</sup>N uptake experiments and harvest*

17  
18 201 | Eight weeks after commencing ~~of~~ the N treatments (and ten weeks after planting), the  
19  
20 202 net inorganic (i.e. ammonium, nitrate) and organic (i.e. glutamine, arginine) N uptake  
21  
22 203 capacity of fine roots of tree seedlings and grasses was quantified following the <sup>15</sup>N  
23  
24 204 enrichment technique described by Gessler et al. (1998) as modified by Simon et al. (2010)  
25  
26 205 using fine roots still attached to the individuals. For this, we carefully removed the plants  
27  
28 206 from the pots and washed the roots to remove adherent soil particles. Fine roots were then  
29  
30 207 incubated for a 2 h period in 4 ml artificial nutrient solution containing all four N sources (as  
31  
32 208 described above), but with one of the sources labelled as either <sup>15</sup>N-ammonium, <sup>15</sup>N-nitrate,  
33  
34 209 <sup>13</sup>C/<sup>15</sup>N-glutamine or <sup>13</sup>C/<sup>15</sup>N-arginine. Amino acids were <sup>13</sup>C/<sup>15</sup>N double-labelled to  
35  
36 210 | determine whether they ~~were~~ taken up as intact molecules or degraded over the time of  
37  
38 211 incubation (Simon et al. 2011). Additionally, we used artificial soil solution without <sup>15</sup>N-label  
39  
40 212 to account for the natural abundance of <sup>15</sup>N in the roots of each species for all treatment  
41  
42 213 levels. Plants were incubated in either low or high N artificial soil solution matching the soil  
43  
44 214 N availability treatment to which they had been subjected. Root incubation occurred between  
45  
46 215 10 am and 2 pm to avoid diurnal variation in N uptake (Gessler et al. 2002), i.e. the incubation  
47  
48 216 period of 2 h for any given individual sample started between 10 am and 12 pm and ended  
49  
50 217 between 12 and 2 pm. For the duration of the <sup>15</sup>N incubation experiments, the remaining  
51  
52 218 seedling's roots were carefully wrapped in wet tissue to prevent drying out. A total of 6  
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54 219 replicate root samples were set up for each of the four N sources (plus controls for natural  
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220 abundance) per species, competition regime and soil N availability level. Depending on the  
 221 size of the root system for an individual plant, one to four N sources plus control were tested  
 222 per individual. After the incubation period, fine roots were carefully cut, washed twice in 0.5  
 223 M CaCl<sub>2</sub> solution to remove the incubation solution from the root surface, and dried with  
 224 tissue. Following the <sup>15</sup>N uptake experiments, we separated the plants into above- and  
 225 belowground organs, i.e. the tree seedlings into leaves, stem and roots, and the grasses into  
 226 shoots and roots, since no stem had developed. We determined the fresh weight (fw) of all  
 227 plant tissues and the incubated fine roots, oven dried the samples at 65°C for 72 hours and  
 228 determined their dry weight (dw) to obtain biomass indices.

### 230 *Quantification of total N and C, <sup>15</sup>N and <sup>13</sup>C in fine roots*

231 To quantify <sup>15</sup>N and <sup>13</sup>C enrichment the dried fine roots were ground using a ball mill  
 232 (TissueLyser, Retsch, Haan, Germany). Aliquots of 1.2-2.5 mg of the resulting homogeneous  
 233 fine powder were weighed into 4x6 mm tin capsules (IVA Analysentechnik,  
 234 Meerbusch, Germany), and analysed with an isotope ratio mass spectrometer (Delta V  
 235 Advantage, Thermo Electron, Dreieich, Germany) coupled to an elemental analyser (Euro  
 236 EA, Eurovector, Milan, Italy).  $\Delta$  values were calculated using a laboratory standard  
 237 (acetanilide) that was part of every sequence in intervals, and also used in different weights to  
 238 determine isotope linearity of the system. The laboratory standard was calibrated against  
 239 different international standards from IAEA (Vienna): for <sup>15</sup>N USGS 40 ( $\delta^{15}\text{N}_{\text{Air}} = -4.5 \text{ ‰}$ ),  
 240 IAEA 600 ( $\delta^{15}\text{N}_{\text{Air}} = +1.0 \text{ ‰}$ ), IAEA N2 ( $\delta^{15}\text{N}_{\text{Air}} = +20.3 \text{ ‰}$ ), USGS 41 ( $\delta^{15}\text{N}_{\text{Air}} = +47.6 \text{ ‰}$ ),  
 241 USGS 26 ( $\delta^{15}\text{N}_{\text{Air}} = +53.7 \text{ ‰}$ ), and USGS 32 ( $\delta^{15}\text{N}_{\text{Air}} = +180 \text{ ‰}$ ), and for <sup>13</sup>C IAEA 600  
 242 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -27.77 \text{ ‰}$ ), IAEA 3 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -24.72 \text{ ‰}$ ), and IAEA CH6 ( $\delta^{13}\text{C}_{\text{V-PDB}} = -10.45$ ).  
 243 <sup>15</sup>N results of highly enriched samples were finally corrected with different enriched <sup>15</sup>N  
 244 standards (from 0.437 to 0.734 at %) from Fischer Analysen Instrumente (Leipzig, Germany).  
 245  $\Delta$  values are defined as  $\delta \text{ [‰]} = (R_{\text{SA}} / R_{\text{Std}} - 1) * 1000$ , where R is the ratio of heavy isotope

1  
2  
3 246 against light isotope (i.e.  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ), SA is sample and Std the international  
4  
5 247 reference for the respective element (V-PDB for  $^{13}\text{C}$  and air- $\text{N}_2$  for  $^{15}\text{N}$ ). Every measuring  
6  
7 248 sequence ended with different suitable international and calibrated laboratory standards for  
8  
9 249 final correction. For each of the four N sources, we then calculated net N uptake capacity  
10  
11 250 (nmol N  $\text{g}^{-1}$  fw  $\text{h}^{-1}$ ) based on the incorporation of  $^{15}\text{N}$  into root fresh weight according to  
12  
13 251 Gessler et al. (1998): net N uptake capacity =  $((^{15}\text{N}_l - ^{15}\text{N}_c) * \text{N}_{\text{tot}} * \text{dw} * 10^5) / (\text{MW} * \text{fw} * t)^{-1}$ , where  
14  
15 252  $^{15}\text{N}_l$  and  $^{15}\text{N}_c$  are the atom% of  $^{15}\text{N}$  in labelled ( $\text{N}_l$ ) and control plants ( $\text{N}_c$ , natural abundance),  
16  
17 253 respectively,  $\text{N}_{\text{tot}}$  is the total N percentage, MW is the molecular weight ( $^{15}\text{N}$   $\text{g mol}^{-1}$ ), and t  
18  
19 254 represents the incubation time (120 min). ~~Net uptake capacity of glutamine and arginine were~~  
20  
21  
22 255 ~~lower when estimated b~~Based on  $^{13}\text{C}$  incorporation, net uptake capacity of glutamine and  
23  
24 256 arginine was ~ 60% and ~ 95% respectively, compared to that than when estimated based on  
25  
26 257  $^{15}\text{N}$  incorporation, which indicates (1) the degradation of amino acids in the solution or on the  
27  
28 258 root surface, and/or (2) the respiration of amino acid-derived C inside the roots (Simon et al.  
29  
30  
31 259 2011).

### 260 261 *Statistical analysis*

262 For all species, we tested for differences between treatments for total biomass and  
263 root:shoot ratio as well as net uptake capacity of the four N sources by performing  
264 Permutational ANOVAs (PERMANOVA) based on a Euclidean resemblance matrix between  
265 samples (Anderson et al. 2008). PERMANOVA was chosen as a non-parametric univariate  
266 test equivalent to ANOVA (Anderson et al. 2008). We performed two-way PERMANOVAs  
267 using “competition regime” and “soil N availability” as orthogonal factors. For the tree  
268 species, the “competition regime” factor had three levels: control, competition with  
269 *Pennisetum*, and competition with *Cynodon*. Moreover, for the tree seedlings biomass and  
270 root:shoot ratio analyses, initial size measurements of the tree seedlings were included as a  
271 covariate. For the grass species, the three levels were: control, competition with *Acacia*, and

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3 272 competition with *Podocarpus*. The “soil N availability” factor included two levels: high N  
4  
5 273 and low N for *Acacia*, *Podocarpus* and *Pennisetum*. For the grass *Cynodon*, we performed  
6  
7 274 one-way PERMANOVAs, considering the factor “competition regime” with three levels:  
8  
9 275 control, competition with *Acacia*, and competition with *Podocarpus*, all at low soil N  
10  
11 276 availability as described above. When a significant interaction between factors was found,  
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13 277 *post hoc* PERMANOVA pair-wise comparisons were performed. To test for differences in net  
14  
15 278 N uptake capacity between species, one-way PERMANOVAs were performed on the control  
16  
17 279 data (i.e. no competition), using species as factor, for each N form. To test for preferences in  
18  
19 280 net uptake capacity of the four N sources for a given species, one-way PERMANOVAs were  
20  
21 281 performed, using N source as factor, for each combination of competition regime and soil N  
22  
23 282 availability treatment. All analyses were performed using PRIMER 6.0 with the  
24  
25 283 PERMANOVA+ add-on (PRIMER-E Ltd, Plymouth, UK), and figures were prepared in  
26  
27 284 SigmaPlot 13.0 (Systat Software Inc., San Jose, USA).  
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## 33 286 **Results**

### 35 287 *Effects of competition regime and soil N availability on biomass and root:shoot ratio*

37 288 Native tree seedlings and exotic grasses responded differently in biomass production  
38  
39 289 depending on competition regime and soil N availability (Figure 1). For the tree species,  
40  
41 290 neither *Acacia* nor *Podocarpus* differed significantly in total biomass between single and  
42  
43 291 competing seedlings regardless of soil N availability (Figure 1, Supplementary Table 1).  
44  
45 292 Positive correlations were found for *Acacia* between total biomass and the initial leaf number,  
46  
47 293 for *Podocarpus* between total biomass and both initial leaf number and initial stem length  
48  
49 294 (both *p*-perm < 0.001, data not shown). This indicates that total biomass at the end of the  
50  
51 295 experiment was a function of initial tree seedling size. For the grass *Pennisetum*, total biomass  
52  
53 296 was significantly reduced in competition with *Acacia* or *Podocarpus* compared to the control  
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55 297 at high soil N availability, and it was also significantly reduced at low compared to high soil  
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3 298 N availability irrespective of the competition regime (Figure 1, Supplementary Table 2). For  
4  
5 299 the grass *Cynodon*, competition with *Acacia* significantly reduced total biomass compared to  
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7 300 competition with *Podocarpus* or control (at low soil N availability) (Figure 1, Supplementary  
8  
9 301 Table 2).  
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13 303 Root:shoot ratio was not affected by soil N availability or competition regime for both  
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15 304 tree species as well as competition regimes for the grass *Cynodon* at low soil N availability  
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17 305 (Figure 1). For the grass *Pennisetum*, root:shoot ratio was significantly lower in competition  
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19 306 with *Podocarpus* compared to competition with *Acacia* or controls irrespective of soil N  
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21 307 availability (Figure 1).  
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26 309 *Effects of competition regime and soil N availability on inorganic and organic net N uptake*  
27  
28 310 *capacity*  
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30  
31 311 Inorganic and organic net N uptake capacity of all species increased significantly with  
32  
33 312 high compared to low soil N availability, whereas the responses to competition regime  
34  
35 313 differed depending on the species (Figures 2 and 3, Supplementary Table 3). Inorganic and  
36  
37 314 organic net N uptake capacity of *Acacia* seedlings increased with higher soil N availability  
38  
39 315 regardless of competition regime. At high soil N availability, net ammonium uptake capacity  
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41 316 of *Acacia* seedlings competing with the grass *Pennisetum* increased significantly compared to  
42  
43 317 seedlings growing without competition (Figure 2, Supplementary Table 4), but no change for  
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45 318 nitrate (Figure 2), glutamine-N or arginine-N (Figure 3) was found at either low or high soil N  
46  
47 319 availability. Similarly, inorganic N (Figure 2) and arginine-N (Figure 3) net uptake capacity  
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49 320 of *Podocarpus* increased with high compared to low soil N availability irrespective of  
50  
51 321 competition regime. However, net glutamine-N uptake capacity increased with higher soil N  
52  
53 322 availability only for *Podocarpus* seedlings grown in competition with *Pennisetum* (Figure 3,  
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55 323 Supplementary Table 4). At high soil N availability, competition with *Pennisetum* increased  
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3 324 net glutamine-N uptake capacity of *Podocarpus* seedlings compared to seedlings growing  
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5 325 alone (Figure 3); however, net ammonium, nitrate (Figure 2), and arginine-N (Figure 3)  
6  
7 326 uptake capacity were not affected. For the grass *Pennisetum*, net ammonium and organic N,  
8  
9 327 but not nitrate uptake capacity were significantly higher at high compared to low soil N  
10  
11 328 availability (Figures 2 and 3). For *Pennisetum*, competition regime had no significant effect  
12  
13 329 on organic (Figure 3) and inorganic (Figure 2) net N uptake capacity at either low or high soil  
14  
15 330 N availability. At low soil N availability, nitrate, but not ammonium or organic net N uptake  
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17 331 capacity of the grass *Cynodon* was significantly higher when grown in competition with  
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19 332 *Podocarpus* compared to that with *Acacia*, or control (Figures 2 and 3, Supplementary Table  
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21 333 4).  
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#### 26 335 *Differences between species with regard to inorganic and organic N acquisition*

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28 336 In the absence of interspecific competition (i.e. single grown tree seedlings or grasses  
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30 337 growing in monocultures), species differed in their inorganic and organic N acquisition  
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32 338 strategies depending on the N source: seedlings of *Podocarpus* took up significantly less  
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34 339 nitrate than seedlings of *Acacia* and the grass *Pennisetum*, irrespective of soil N availability  
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36 340 (Table 1). However, glutamine-N acquisition of *Pennisetum* was significantly lower than that  
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38 341 of *Podocarpus*, which in turn was lower than that of *Acacia* seedlings at both low and high  
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40 342 soil N availability (Table 1). No differences were found between any of the species with  
41  
42 343 regard to ammonium and arginine-N acquisition (Table 1).  
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48 345 General preference patterns of the different N forms were found for each species  
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50 346 within each competition regime depending on soil N availability: At low soil N availability,  
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52 347 both tree species preferred nitrate and glutamine-N over ammonium and arginine-N  
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54 348 (Supplementary Table 5). However, at high soil N availability, this pattern was reversed with  
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56 349 ammonium and arginine-N being favoured over nitrate and glutamine-N for both tree species  
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3 350 | ([Table 2](#), Supplementary Table 5). For the grasses, inorganic N sources, particularly nitrate,  
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5 351 | were preferred over organic N sources (regardless of soil N availability for *Pennisetum*)  
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7 352 | ([Table 2](#), Supplementary Table 6).  
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9 353 |

## 11 354 **Discussion**

13 355 | Our study provides novel insight into the species-specific growth and N acquisition  
14 356 | patterns in native tree seedlings and exotic grasses competing for N. Depending on soil N  
15 357 | availability, native tree seedlings responded to competition with increases in N acquisition  
16 358 | only, whereas the responses of the grasses in terms of biomass and N acquisition varied with  
17 359 | species and soil N availability. These results highlight that quantifying underlying  
18 360 | mechanisms complements biomass indices to advance the understanding of interspecific  
19 361 | competition (Trinder et al. 2013). Furthermore, the lack of response with regard to biomass in  
20 362 | the tree seedlings compared to the grasses suggests that N storage capacity plays a role in the  
21 363 | growth response as well.  
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35 365 | *Native tree seedlings increase N acquisition in competition with exotic grasses at high soil N*  
36 366 | *availability*

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39 367 | At high soil N availability, both tree species responded similarly to competition with  
40 368 | the exotic grass *Pennisetum* by increasing N acquisition. However, growth and biomass  
41 369 | allocation were unaffected, which indicates that they might allocate the newly acquired N  
42 370 | to storage rather than investing into growth, thereby reducing N availability for their  
43 371 | competitors (Millard and Grelet 2010). These similarities between *Acacia* and *Podocarpus* in  
44 372 | terms of net N uptake capacity and growth responses to competition with grasses indicate that  
45 373 | both tree species use a common strategy, which is rather unexpected given the different  
46 374 | successional status, growth strategies and physiological characteristics of the two tree species.  
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57 375 | *Acacia* is an early successional, fast-growing and N<sub>2</sub> fixing species, while *Podocarpus* is a  
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3 376 slow-growing, late successional species (Smith et al. 2008). Thus, since fast-growing species  
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5 377 would require higher amounts of N to meet their growth rate (Poorter et al. 2012), the  
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7 378 observed increase in net N uptake capacity when growing in competition would be expected  
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9 379 in *Acacia* and not in *Podocarpus*. However, previous studies have shown that *Acacia*  
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11 380 seedlings are capable of N-fixation (Sun et al. 1992), and that root nodulation in *Acacia*  
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13 381 species is stimulated by competition with grasses (Cramer et al. 2007), ~~and t~~Therefore the  
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15 382 increased requirements of *Acacia* due to competition would probably be mitigated by its  
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17 383 ability to fix atmospheric N<sub>2</sub> (not quantified in our study) and thus reduce to an extent its  
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19 384 dependency on external N supply. Moreover, the lack of response of *Acacia* to competition in  
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21 385 terms of N uptake at low soil N availability in our study might also be attributed to a greater  
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23 386 reliance on N<sub>2</sub> fixation under limiting N conditions (Pfautsch et al. 2009).  
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30 388 Tree species differed in the acquired N forms: in competition fast-growing *Acacia*  
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32 389 acquired more ammonium compared to single grown individuals, while slow-growing  
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34 390 *Podocarpus* used glutamine-N. This preference of inorganic vs. organic N forms when  
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36 391 competing might reflect different N acquisition strategies between fast- and slow-growers as  
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38 392 reported for temperate tree seedlings (Simon et al. 2010, Simon et al. 2014, Li et al. 2015).  
39  
40 393 The similar responses found here might be an early indication that, across biomes, N  
41  
42 394 acquisition of trees is linked to growth strategy. These increases in net N uptake capacity as a  
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44 395 response to competition were found only at high soil N availability suggesting mediation of N  
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46 396 acquisition by soil N availability (e.g. Simon et al. 2013, Li et al. 2015). For *Acacia*, this  
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48 397 could be related to a reduced nodule activity at high soil N concentrations, as it has been  
49  
50 398 suggested that N acquisition from the soil, rather than N<sub>2</sub> fixation, is energetically more  
51  
52 399 efficient for this species when inorganic N is readily available (Pfautsch et al. 2009). With  
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54 400 regard to *Podocarpus*, an increase in organic N acquisition with competition was also  
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3 401 reported for a temperate slow-growing tree species at high, but not low soil N (Li et al. 2015).  
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5 402 Our results constitute a further example of increased N acquisition under competition with  
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7 403 high N availability, but in a subtropical environment. Particularly in the view of increased  
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9 404 atmospheric N deposition in the future (Rennenberg and Dannenmann 2015, [Jia et al. 2016](#)),  
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11 405 the outcomes of competition in currently N-limited habitats are likely to change.  
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17 407 At low soil N availability, competition with grasses did not result in changes in N  
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19 408 acquisition or biomass production and allocation for both tree species. Similarly, previous  
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21 409 studies have reported a lack of effects of competition with the grass *Cynodon dactylon* on the  
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23 410 growth (i.e. biomass, basal diameter and stem height) of a different *Acacia* species (*A. smallii*  
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25 411 Isely) on non-supplemented native soil (Cohn et al., 1989). Nevertheless, this grass species  
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27 412 hinders the growth of native tree seedlings in the field and is reported as a threat to restoration  
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29 413 efforts in subtropical Australia (M. Amos, pers. comm.). Since we did not observe these  
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31 414 negative effects in our study, it is likely that the impacts of the competition with *Cynodon* on  
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33 415 tree seedlings growth are evident at later developmental stages of both the grass and the tree  
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35 416 species than the ones considered here. The lack of response to competition of the tree  
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37 417 seedlings regarding total biomass could also be due to the short duration of our experiment,  
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39 418 considering the relatively slow growth of tree seedlings compared to grasses. Previous studies  
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41 419 reporting negative effects of grasses on *Acacia* measured responses over longer periods, e.g. 3  
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43 420 years (Messina and Barton 1985), and 8 years (Florentine and Westbrooke 2004).  
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45 421 Furthermore, the effect of the grass on tree seedlings may be and/or that they are mediated by  
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47 422 soil N availability. In our study, the effects of competition with *Cynodon* were not  
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49 423 investigated at high soil N availability, but it has been reported that competition with *Cynodon*  
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51 424 combined with an increase in soil nutrient availability results in 70-90% reduction in growth  
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53 425 of *A. smallii* (Cohn et al., 1989).  
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427 *Exotic grasses respond to competition for N depending on the competitor and soil N*  
428 *availability*

429 For the exotic grasses, four strategies were identified which depended on the  
430 competing species and soil N availability: (1) *Pennisetum* biomass was reduced in  
431 competition with both tree species compared to the control at high, but not low soil N  
432 availability suggesting that the outcome of competitive interactions between native tree  
433 seedlings and *Pennisetum* depends on soil N availability. Moreover, the biomass of  
434 *Pennisetum* at high soil N availability, even if reduced by competition with the tree seedlings,  
435 was still higher than its biomass at low soil N in the absence of interspecific competition,  
436 suggesting that, at least in the short-term, the grass is more successful than the tree seedlings  
437 at high soil N availability, despite the observed negative effects of competition (2) The grass  
438 *Cynodon* showed a similar strategy as *Pennisetum*, i.e. a reduction in biomass but no change  
439 in N acquisition, but only when competing with *Acacia*, but not *Podocarpus*, indicating that  
440 for both grass species the response to competition depends on the competitor. (3) Resource  
441 allocation shifted to aboveground at the expense of belowground tissue production (Aerts et  
442 al. 1991) for *Pennisetum* competing with *Podocarpus* at low and high soil N. This suggests  
443 that, despite the fact that shading from the tree seedlings was not apparent, the potential  
444 competition for light is more relevant for *Pennisetum* than competition for belowground  
445 resources (Poorter et al. 2012), thus not leading to an increase in N acquisition. However,  
446 these results were not observed when grown in competition with *Acacia* or for the grass  
447 *Cynodon* indicating that this biomass allocation strategy of *Pennisetum* also depends on the  
448 competing species, but not soil N availability. (4) Competition with *Podocarpus*, but not  
449 *Acacia*, resulted in increased net nitrate uptake in *Cynodon*, but not *Pennisetum* suggesting  
450 different N acquisition strategies between the two exotic grass species that vary with the  
451 competing tree species. Overall, our results suggest that the outcome of competitive

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3 452 interactions between native tree seedlings and exotic grasses are species-specific and  
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5 453 mediated by soil N availability.

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9 455 *Differences in N acquisition strategies between species suggest potential for niche*  
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11 456 *differentiation*

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13 457 All four species took up both inorganic and organic N forms, although specifics varied  
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15 458 according to N forms. Nitrate acquisition was higher in *Pennisetum* and *Acacia* compared to  
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17 459 *Podocarpus*, consistent with their successional status and the low abundance of nitrate in late-  
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19 460 successional forest soils as well as a reduced nitrate use capacity in late-successional tree  
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21 461 species (Kronzucker et al. 1997, Britto and Kronzucker 2013). At sites with higher soil nitrate  
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23 462 availability, e.g. disturbed areas, the grass *Pennisetum* would have a competitive advantage  
24  
25 463 over *Podocarpus*, but not *Acacia*, highlighting the importance of species-specific  
26  
27 464 consideration for reforestation programs. Moreover, glutamine-N acquisition was higher for  
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29 465 *Acacia* than *Podocarpus*, contrary to what we expected according to their successional status,  
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31 466 since soil amino acid content generally increases as succession progresses (Werdin-Pfisterer  
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33 467 et al. 2009). Nevertheless, glutamine-N acquisition was higher in both tree species compared  
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35 468 to *Pennisetum* indicating a potential for avoidance of competition via the use of the different  
36  
37 469 N forms (Simon et al. 2017). Overall, that plants take up organic N forms is now well  
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39 470 established and has been reported for numerous plant species and ecosystems (Näsholm et al.  
40  
41 471 2009). Moreover, its ecological relevance is increasingly recognised due to the resulting niche  
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43 472 differentiation and consequences in terms of species coexistence (Näsholm et al. 2009, Simon  
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45 473 et al. 2014, Li et al. 2015, Andersen et al. 2017, Uscola et al. 2017).

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49 475 Comparing within tree and grass species, higher soil N availability resulted in  
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51 476 increased inorganic and organic N acquisition in general, which for *Pennisetum* was  
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53 477 combined with increased biomass production (for *Cynodon* data is only available for low soil  
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3 478 N). This increase in grass biomass production with increasing soil N availability while there  
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5 479 was no short-term response of the tree seedlings suggests a potential advantage for the grass  
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7 480 in habitats on N-rich soils or in future scenarios with increased atmospheric N deposition  
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9 481 compared to the native trees. This link between high resource availability and success of  
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11 482 invasive species has been explored in previous studies (e.g. Badgery et al. 2005, Gao et al.  
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13 483 2014, Yelenik et al. 2017), and has been linked to invader traits related to exploitation and  
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15 484 efficient use of resources, which allows for better growth (Huangfu et al. 2016, Yelenik et al.  
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17 485 2017). In our study, although competition with seedlings of both tree species reduced the total  
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19 486 biomass of *Pennisetum* compared to *Pennisetum* grown in controls, the increase in  
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21 487 *Pennisetum* biomass in response to higher soil N availability was of greater magnitude.  
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23 488 Increases in the productivity of *Pennisetum* with N fertilization resulting in its dominance at  
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25 489 fertilised sites have been reported before (Mears 1970, Barton et al. 2008), and under such  
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27 490 conditions, *Pennisetum* might be more competitive than tree seedlings. Moreover, the  
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29 491 potential of *Acacia* to induce increases in soil N availability over time as a consequence of its  
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31 492 N<sub>2</sub> fixing ability has been shown before in silvopastoral systems (Power et al. 2003). Thus,  
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33 493 increased N supply as a consequence of the use of N<sub>2</sub> fixing tree species such as *Acacia* might  
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35 494 favour the growth and potential dominance of *Pennisetum* at restoration sites.  
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#### Methodological considerations

Our experimental approach has several limitations. First, the competitor grasses were grown from seeds, whereas the trees were purchased as seedlings. This set up was selected because our aim was to mimic the actual timing in a restoration setting under field conditions for which tree species are planted as seedlings for reforestation and grasses arrive at a later time. Thus, a comparison between trees and grasses cannot exclude differences due to variation in developmental stages between tree species and grasses. Specifically, the lack of response in seedling biomass reported here might not persist with older or larger grasses.

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3 504 Second, tree seedlings differed in their age at the start of the experiment which might have  
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5 505 influenced their responses to competition and/or soil N availability in our experiment.  
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7 506 However, given the differences in growth rates between the tree species used in this study,  
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9 507 seedlings of similar age would have likely differed in their initial above- and belowground  
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11 508 dimensions, thereby introducing another variable. Third, the experiment ran for eight weeks  
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13 509 which might not be sufficient time for a response in tree seedling biomass, however they did  
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15 510 respond with regard to N acquisition from the soil. Finally, the artificial N solution used in  
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17 511 this study is based on a low and high N forest site (Dannenmann et al. 2009, Stoelken et al.  
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19 512 2010, respectively) as well as the occurrence of glutamine and arginine in soils of subtropical  
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21 513 Queensland (Holst et al. 2012), but not actual measured concentrations at subtropical  
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23 514 restoration sites.  
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## 29 516 *Conclusions*

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31 517 In our study, native tree seedlings and exotic grasses responded to competition with  
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33 518 two distinct response types: (1) a reduction in biomass, but no change in N acquisition, (2) an  
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35 519 increase in N acquisition without a change in biomass. This outcome depended on the  
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37 520 competing species and soil N availability. Thus, we suggest that these aspects be considered  
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39 521 also for ecosystem restoration and management, with special attention to the potential  
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41 522 feedbacks between native trees possessing traits such as N<sub>2</sub> fixing ability and exotic grasses  
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43 523 that may benefit from the resulting increase in soil N availability. Exotic grasses such as  
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45 524 *Pennisetum* and *Cynodon* are considered a high priority for control in Australia and their  
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47 525 suppression can promote the reestablishment of woody vegetation, thus determining the  
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49 526 success of subtropical rainforest restoration programs (Florentine and Westbrooke 2004,  
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51 527 Downey et al. 2010, Shoo and Catterall 2013). We found no common N acquisition strategy  
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53 528 across species in response to competition between native trees and invasive grasses indicating  
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55 529 the need to consider species-specific approaches in restoration management plans.  
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**779 Figure legends**

780 Figure 1. Total biomass and root:shoot ratio of *Acacia melanoxylon*, *Podocarpus elatus*,  
781 *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability under  
782 different competition regimes. For *C. dactylon*, only low soil N data is available. comp  
783 *Pennisetum* = competition with *P. clandestinum*; comp *Cynodon* = competition with *C.*  
784 *dactylon*; comp *Acacia* = competition with *A. melanoxylon*; comp *Podocarpus* = competition  
785 with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters  
786 indicate significant differences between competition regimes within a specific soil N  
787 availability treatment, and asterisks indicate significant differences between soil N availability  
788 treatments, detected using permutational analysis of variance ( $p < 0.05$ ).

789

790 Figure 2. Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) net uptake capacity ( $\text{nmol N g}^{-1} \text{fw h}^{-1}$ ) by  
791 fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon*  
792 *dactylon* at low and high soil N availability under different competition regimes. For *C.*  
793 *dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P.*  
794 *clandestinum*; comp *Cynodon* = competition with *C. dactylon*; comp *Acacia* = competition  
795 with *A. melanoxylon*; comp *Podocarpus* = competition with *P. elatus*. Box plots show mean  
796 (dotted line) and median (straight line). Different letters indicate significant differences  
797 between competition regimes within a specific soil N availability treatment, and asterisks  
798 indicate significant differences between soil N availability treatments detected using  
799 permutational analysis of variance ( $p < 0.05$ ).

800

801 Figure 3. Glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity ( $\text{nmol N g}^{-1} \text{fw h}^{-1}$ )  
802 by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and  
803 *Cynodon dactylon* at low and high soil N availability under different competition regimes. For  
804 *C. dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P.*

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3 805 *clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition  
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5 806 with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean  
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7 807 (dotted line) and median (straight line). Different letters indicate significant differences  
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9 808 between competition regimes within a specific soil N availability treatment, and asterisks  
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11 809 indicate significant differences between soil N availability treatments detected using  
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13 810 permutational analysis of variance ( $p < 0.05$ ).  
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Table 1. PERMANOVA results of the differences in ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots between *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*.

	NH <sub>4</sub> <sup>+</sup>		NO <sub>3</sub> <sup>-</sup>		Gln-N		Arg-N	
<i>Main test</i>	<i>Pseudo-F</i>	<i>P(perm)</i>	<i>Pseudo-F</i>	<i>P(perm)</i>	<i>Pseudo-F</i>	<i>P(perm)</i>	<i>Pseudo-F</i>	<i>P(perm)</i>
Species	1.630	0.170	4.248	<b>0.013</b>	7.469	<b>&lt;0.001</b>	0.573	0.648
Soil N availability	16.749	<b>&lt;0.001</b>	0.674	0.419	8.259	<b>0.006</b>	65.941	<b>&lt;0.001</b>
Species x Soil N availability	2.184	0.098	0.011	0.991	2.545	0.092	1.307	0.295
<i>Pairwise comparisons</i>	<i>t</i>	<i>P(perm)</i>	<i>t</i>	<i>P(perm)</i>	<i>t</i>	<i>P(perm)</i>	<i>t</i>	<i>P(perm)</i>
<i>Acacia vs Podocarpus</i>	Not applicable		3.843	<b>&lt;0.001</b>	2.493	<b>0.022</b>	Not applicable	
<i>Acacia vs Pennisetum</i>			1.180	0.251	4.766	<b>&lt;0.001</b>		
<i>Acacia vs Cynodon</i>			0.803	0.441	1.771	0.096		
<i>Podocarpus vs Pennisetum</i>			3.0987	<b>0.005</b>	2.207	<b>0.041</b>		
<i>Podocarpus vs Cynodon</i>			1.760	0.066	1.401	0.179		
<i>Pennisetum vs Cynodon</i>			1.125	0.271	1.509	0.157		

Table 2. Differences between ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity of fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability. Only significant differences are presented.

	<i>Acacia</i>	<i>Podocarpus</i>
<b>1) Low soil N availability</b>		
Control	$\text{NO}_3^-$ , Gln-N > $\text{NH}_4^+$ > Arg-N	Gln-N > $\text{NH}_4^+$ , $\text{NO}_3^-$ , Arg-N $\text{NO}_3^-$ > Arg-N
Competition with <i>Pennisetum</i>	$\text{NO}_3^-$ , Gln-N > $\text{NH}_4^+$ , Arg-N	$\text{NO}_3^-$ , Gln-N > $\text{NH}_4^+$ , Arg-N
Competition with <i>Cynodon</i>	Gln-N > $\text{NH}_4^+$ , Arg-N	Gln-N > $\text{NO}_3^-$ , Arg-N > $\text{NH}_4^+$
<b>2) High soil N availability</b>		
Control	No differences	$\text{NH}_4^+$ > $\text{NO}_3^-$ , Gln-N, Arg-N
Competition with <i>Pennisetum</i>	$\text{NH}_4^+$ > $\text{NO}_3^-$ , Gln-N, Arg-N Arg-N > Gln-N	$\text{NH}_4^+$ > $\text{NO}_3^-$ , Gln-N, Arg-N Gln-N > $\text{NO}_3^-$
	<b><i>Pennisetum</i></b>	<b><i>Cynodon</i>*</b>
<b>1) Low soil N availability</b>		
Control	$\text{NO}_3^-$ > $\text{NH}_4^+$ , Gln-N, Arg-N	$\text{NO}_3^-$ , Gln-N > $\text{NH}_4^+$
Competition with <i>Acacia</i>	$\text{NO}_3^-$ > $\text{NH}_4^+$ , Gln-N, Arg-N Gln-N > Arg-N	$\text{NO}_3^-$ > $\text{NH}_4^+$
Competition with <i>Podocarpus</i>	$\text{NO}_3^-$ > $\text{NH}_4^+$ , Arg-N $\text{NH}_4^+$ , Gln-N > Arg-N	$\text{NO}_3^-$ > $\text{NH}_4^+$ , Gln-N, Arg-N Gln-N > Arg-N
<b>2) High soil N availability</b>		
Control	$\text{NH}_4^+$ > $\text{NO}_3^-$ , Gln-N, Arg-N Arg-N > Gln-N	
Competition with <i>Acacia</i>	$\text{NH}_4^+$ > Arg-N > Gln-N $\text{NO}_3^-$ > Gln-N	
Competition with <i>Podocarpus</i>	$\text{NH}_4^+$ > Arg-N > Gln-N	

\* at low soil N availability

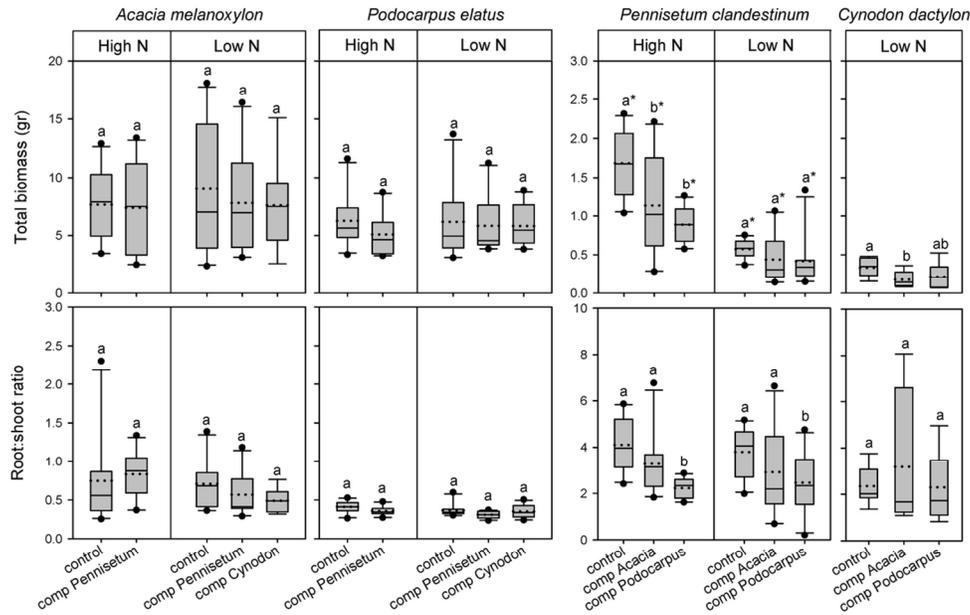


Figure 1. Total biomass and root:shoot ratio of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon*, at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp Pennisetum = competition with *P. clandestinum*; comp Cynodon = competition with *C. dactylon*; comp Acacia = competition with *A. melanoxylon*; comp Podocarpus = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments, detected using permutational analysis of variance ( $p < 0.05$ ).

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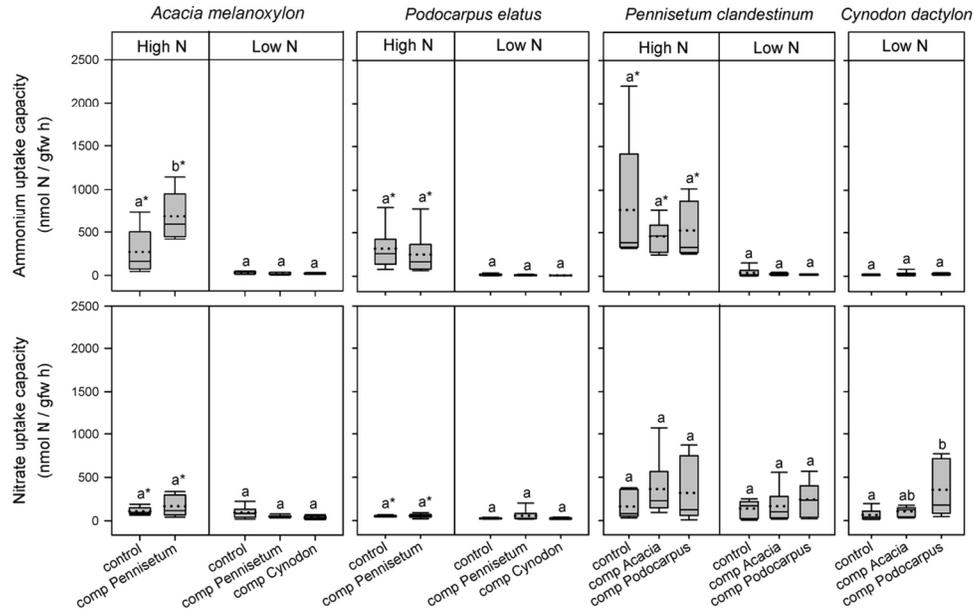


Figure 2. Ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) net uptake capacity (nmol N g<sup>-1</sup> fw h<sup>-1</sup>) by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon* at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P. clandestinum*; comp *Cynodon* = competition with *C. dactylon*; comp *Acacia* = competition with *A. melanoxylon*; comp *Podocarpus* = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ( $p < 0.05$ ).

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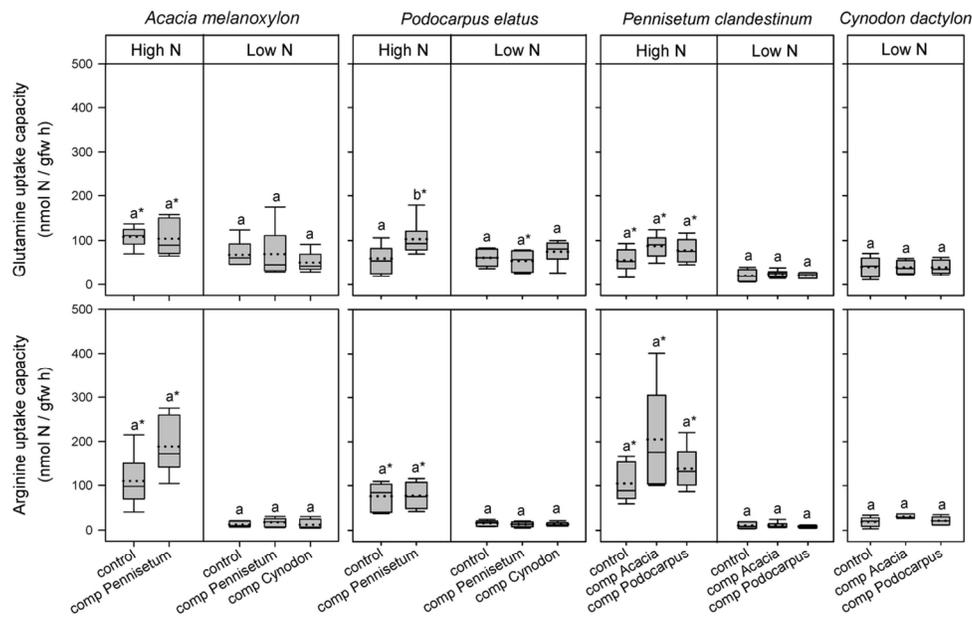


Figure 3. Glutamine-N (Gln-N) and arginine-N (Arg-N) net uptake capacity (nmol N g<sup>-1</sup> fw h<sup>-1</sup>) by fine roots of *Acacia melanoxylon*, *Podocarpus elatus*, *Pennisetum clandestinum* and *Cynodon dactylon* at low and high soil N availability under different competition regimes. For *C. dactylon*, only low soil N data is available. comp *Pennisetum* = competition with *P. clandestinum*; comp *Cynodon* = competition with *C. dactylon*; comp *Acacia* = competition with *A. melanoxylon*; comp *Podocarpus* = competition with *P. elatus*. Box plots show mean (dotted line) and median (straight line). Different letters indicate significant differences between competition regimes within a specific soil N availability treatment, and asterisks indicate significant differences between soil N availability treatments detected using permutational analysis of variance ( $p < 0.05$ ).

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