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Exceptional nitrogen-resorption efficiency enables *Maireana* species (Chenopodiaceae) to function as pioneers at a mine-restoration site

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1 Abstract

Tailings are among the most challenging mined substrates for plant re-establishment, in particular because of a lack of soil-like structure and nitrogen. Potential pioneer plants are sometimes found at such disturbed and infertile sites. We present a group of pioneer species in the genus *Maireana* (Chenopodiaceae) that are promising candidates for the restoration of magnetite tailings. We found that these *Maireana* species did not rely on biologically fixed N from the atmosphere, but exhibited an exceptionally high leaf N-resorption efficiency (about 95%) during leaf senescence, at the same time effectively scavenging trace amount of N from the substrate, in part through rapid transpiration.

2 Introduction

Australia is globally one of the largest producers and exporters of iron (Fe) ores (Senior et al., 2020). Iron-ore mining activities in Western Australia occur in areas harbouring high plant species diversity with high rates of endemism (Gibson et al., 2012). It is a legislated requirement in regions such as Western Australia that post-mining landscapes are restored to the native plant community as part of mine-site closures (Stevens and Dixon, 2017). The edaphic conditions of mined waste tailings are usually very challenging for plant growth, both physically and chemically (Cross and Lambers, 2017). A lack of nitrogen (N) is a major constraint for plant growth in Fe-ore tailings (Cross et al., 2019). Various strategies have been explored to facilitate the revegetation of tailings *in situ* including the use of pioneer plants. It is common for pioneer species to be found as natural early colonisers at the boundary of or even on highly disturbed sites (Arocena et al., 2010). A high N-resorption efficiency is one of the key traits for plant species to establish in such infertile disturbed soils (Richardson et al., 2005). This short communication reports potential candidates of pioneer species in the genus *Maireana* (Chenopodiaceae), for restoration of magnetite tailings and document why these species are successful on the tailings. The questions investigated include: (1) do *Maireana* species access atmospheric N in association with endosymbionts; and (2) are *Maireana* species particularly efficient at remobilising N from senescing leaves.

3 Material and Methods

3.1 Study area

The study site was located in a magnetite mining operation in the Mid-west region of Western Australia, approximately 400 km northeast of Perth. This area experiences a semi-arid dry Mediterranean climate with mild winters (mean monthly maximum 19 °C) and hot, dry summers (mean monthly maximum 37 °C). The study area receives an average of 311 mm annual rainfall, about 65% of which falls in the winter (May – September) (Australian Bureau of Meteorology, http://www.bom.gov.au/climate/data/). Plant communities are generally low to open woodlands, predominantly comprising shrubs or trees of *Acacia* spp. and *Eucalyptus* spp., with an understorey of shrubs, grasses and herbaceous annuals (Markey and Dillon, 2008). Natural soils at the study site comprise highly-weathered red earth, from stony red loamy sand to loamy clay soil. Soil and leaf samples were taken for analysis in September 2019.

Two batches of leaf samples were collected, including plants (*Eucalyptus loxophleba*, *Acacia assimilis*, and five *Maireana* species) in the undisturbed natural vegetation and three *Maireana* species on the magnetite tailings. *Eucalyptus loxophleba* (non-nitrogen (N)-fixing) and *A. assimilis* (N-fixing species) were selected as negative and positive references, respectively, to assess whether *Maireana* species had access to biologically-fixed N. Foliar δ^{13} carbon (C) and δ^{15}N were determined to assess plant water-use efficiency and photosynthetic pathway (C_3, C_4 or CAM; Lambers and Oliveira 2019) and reliance on biologically-fixed N (Vitousek et al. 2013). Soil samples were collected from five reference sites, air-dried and 2-mm sieved, followed by analyses of a range of soil chemical properties. Statistical analyses were performed using one-way ANOVA or Kruskal-Wallis test in JMP® 15. More details on methods of leaf, soil and statistical analyses are provided in the Supplemental Materials.

4 Results

Tailings were highly alkaline, very low in organic carbon (C) concentration, and did not contain detectable concentrations of N, compared with natural topsoils (Table 1). Tailings had about 10 times more total P than natural topsoil. Colwell P, measured as a potentially plant-available P fraction, was similar in natural topsoil and tailings.

Table 1 Selected chemical properties of natural reference soils at the mine site. Data are mean values with standard error in parentheses (n=5); values are expressed on a dry soil basis. EC: electrical conductivity. "–" means data no available. Different letters in a row indicate significant differences ($p < 0.05$).

	Natural soil	Magnetite tailings[1]
pH (H_2O)	5.7 (0.2)[b]	10.0 (0.1)[a]
pH ($CaCl_2$)	4.9 (0.1)[b]	8.7 (0.1)[a]
EC (mS m^{-1})	8 (1)[b]	25 (4)[a]
Organic C (mg g^{-1})	8 (1)[a]	2 (1)[b]
Inorganic C (mg kg^{-1})	27 (8)	-
Total N (mg kg^{-1})	626 (195)	Not detected
Mineral N (mg kg^{-1})	8.5 (3.5)	Not detected
Total P (mg kg^{-1})	139 (10)[b]	1149 (22)[a]
Inorganic P (mg kg^{-1})	54 (4)[b]	1124 (24)[a]
Organic P (mg kg^{-1})	85 (7)[a]	24 (2)[b]
Colwell P (mg kg^{-1})	9.2 (0.8)[a]	9 (1)[a]
Organic P/Total P (%)	61 (2)[a]	2.1 (0.2)[b]
Colwell P/Total P (%)	6.6 (0.4)[a]	0.8 (0.1)[b]

[1] Data of pH (H_2O), EC, organic C, total N, and mineral N were sourced from Cross et al. (2018.

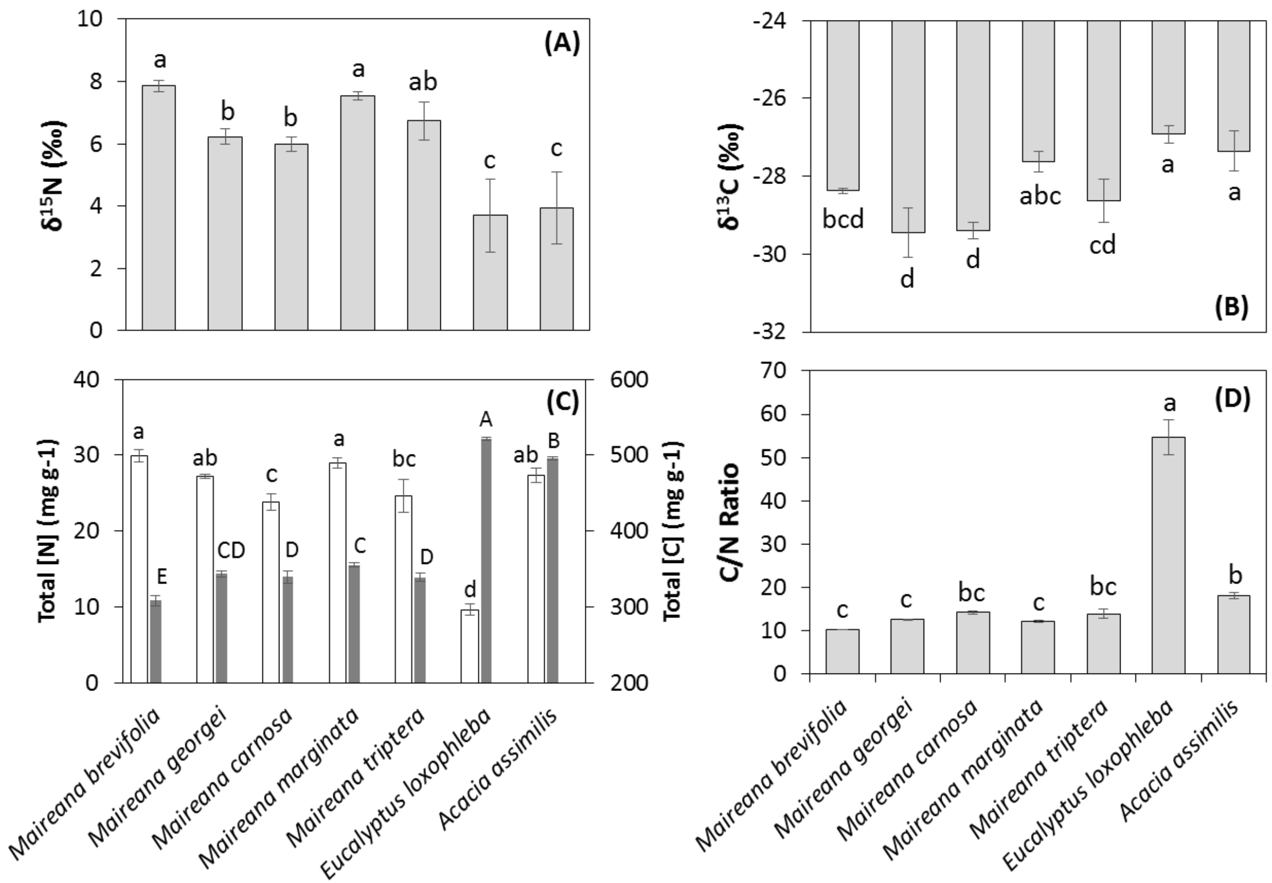

Figure 1. (A) $\delta^{15}N$, (B) $\delta^{13}C$, (C) total nitrogen (N) (empty bars) and carbon (C) (grey bars) concentrations, and (D) C/N ratios in leaves of selected species found at natural sites. Data are mean values ± standard error (n=5). Different letters indicate significant differences (p < 0.05).

In the natural habitats, all *Maireana* species had very positive $\delta^{15}N$ natural abundance values (greater than 6‰), which were significantly greater than those of *E. loxophleba* and *A. assimilis* (about 4‰) (Figure 1A). However, *E. loxophleba* and *A. assimilis* had significantly less negative $\delta^{13}C$ values (about -27‰) than four of the *Maireana* species (*M. brevifolia*, *M. georgei*, *M. carnosa*, and *M. triptera*) (between -28 to -30‰) (Figure 1B); but not *M. marginata*. The $\delta^{13}C$ values indicated that all species exhibited the C_3 photosynthetic pathway (Lambers and Oliveira 2019). All *Maireana* species had similar foliar [N] as *A. assimilis* (close to 30 mg g^{-1}), which was almost three times higher than that of *E. loxophleba* (Figure 1C). The total [C] of *E. loxophleba* and *A. assimilis* were significantly higher than those of all *Maireana* species (Figure 1C). *Eucalyptus loxophleba* had significantly higher C/N ratio than the other species (Figure 1D).

In the tailings storage facility, foliar [N] showed a similar pattern in mature and senesced leaves of three *Maireana* plants. All three *Maireana* plants had about 95% of N-adsorption efficiency during senescence (Figure 2).

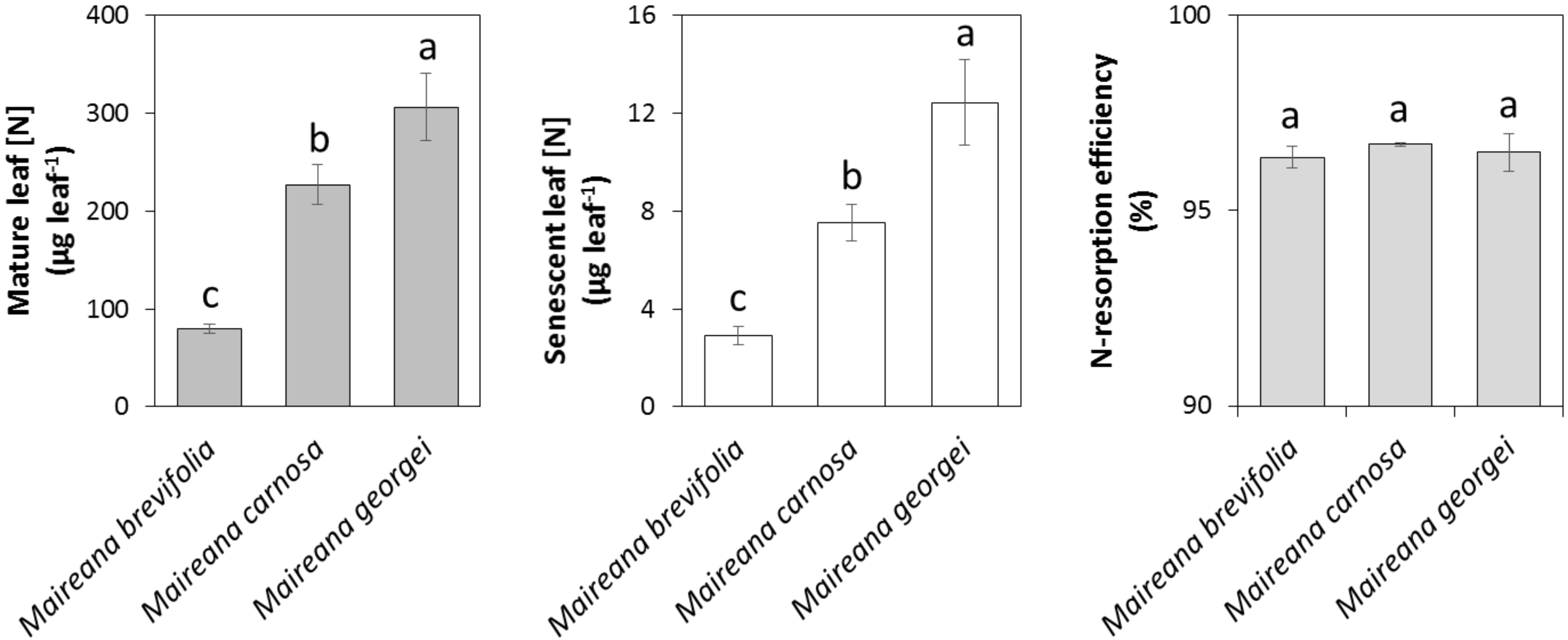

Figure 2. Nitrogen (N) content in mature and senesced leaves, and N-resorption efficiency of three *Maireana* species occurring on the tailings storage facility. Data are mean values ± standard error (n=3). Same letters indicate no significant differences ($p < 0.05$).

5 Discussion

Maireana species are successful pioneer plants on tailings storage facilities, both on a tailings dam at mine site and in glasshouse trials (Cross et al., 2021). However, it is puzzling how *Maireana* species can grow in pure tailings, since tailings did not contain detectable levels of N (Table 1). Our leaf $\delta^{15}N$ results provided no evidence that *Maireana* species accessed biologically fixed atmospheric N in their natural habitat, because the $\delta^{15}N$ values were all much higher than those of the N-fixing species *A. assimilis* (Fabaceae) and the non-N-fixing *E. loxophleba* (Myrtaceae). The similarity in leaf $\delta^{15}N$ values in *A. assimilis* and *E. loxophleba*, with no evidence of nodulation in multiple field investigations, suggests little or no input of fixed atmospheric N by *A. assimilis* in the present study. This is consistent with the findings of Pate et al. (1998), who suggested that *Acacia* species are rather conservative in performing N_2 fixation in the dry Australian mulga ecosystems consisting of a complex of closely related *Acacia* species. In contrast, Wu et al. (2021) found that establishment of *M. brevifolia* on aged magnetite tailings drove the development of prokaryotic microbial communities, suggesting the potential of *M. brevifolia* to access N fixed by free living N-fixing bacteria. However, our findings of leaf $\delta^{15}N$ are inconsistent with their suggestion. It is likely that N-fixing microbes have not yet arrived in the tailings.

Because our $\delta^{15}N$ values indicated that *Maireana* species did not rely on N fixed by endosymbionts, we then explored alternative possibilities. First, we considered if *Maireana* species might depend on N sourced from N deposition? We concluded that this is very minor at most, since the average N-deposition rate in the region is only 1-2 kg N ha^{-1} yr^{-1} (Ochoa-Hueso et al., 2011). Second, we assessed if growth of the present *Maireana* species might be sustained by relatively high seed N reserves. We concluded that this is also unlikely because the mean seed [N] of the selected *Maireana* species is only 23.7 mg g^{-1}, and their mean dry seed mass is only 7.7 mg $seed^{-1}$ (A.T. Cross unpubl.). The leaf $\delta^{13}C$ values of the *Maireana* species indicate that they exhibited a low water-use efficiency, compared with *E. loxophleba* and *A. assimilis* (Figure 1B). So, it is possible that by transpiring rapidly when water is available, *Maireana* species can acquire the small amount of N in the soil via mass flow towards the roots and scavenge what little N is present. A similar mechanism has been reported in a plant mesocosm study using low-N fertility soils (Matimati et al., 2014).

We found that all three *Maireana* species effectively remobilised N from senescing leaves, showing an N-resorption efficiency of about 95% (Figure 2). This is much higher than that of plant species on soils of the youngest stage of a sand dune chronosequence in south-western Australia, where N limits plant productivity (45% of N-resorption efficiency) (Hayes et al., 2014). The present value of 95% is also much greater than the global average N-resorption efficiency of 62% (ranging from 56% to 75%) (Vergutz et al., 2012).

In summary, we surmise that scavenging trace amount of N from the soil, in part through rapid transpiration, and exhibiting a very high leaf N-resorption efficiency, are the most likely reasons why the present *Maireana* species are successful on magnetite tailings at the mine site.

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