Foliar nitrogen dynamics of an invasive legume compared to native non-legumes in fynbos riparian zones varying in water availability

Casparus J Crous[1,4]*, Deanne C Drake[2], Anna L Jacobsen[3], R Brandon Pratt[3], Shayne M Jacobs[4] and Karen J Esler[4,5]
[1]South African Environmental Observation Network, Arid Lands Node, P.O. Box 110040, Hadison Park 8306, Kimberley, South Africa
[2]Wisconsin Department of Natural Resources, USGS Upper Midwest Environmental Science Center, 2630 Fanta Reed Rd, La Crosse, WI, 54603, USA
[3]Department of Biology, California State University, Bakersfield, 9001 Stockdale Hwy, Bakersfield, CA, 93311, USA
[4]Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa
[5]Centre for Invasion Biology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

ABSTRACT

The legume *Acacia mearnsii* invades South Africa's fynbos riparian zones and may alter the nitrogen (N) dynamics and supply in these areas that typically support few native N fixers. Nitrogen uptake by *A. mearnsii* may also be influenced by water availability, potentially affecting riparian-specific performance and impact estimations. We expected to find functional differences between the invasive legume and the two co-occurring but non-leguminous native species *Brabejum stellatifolium* and *Metrosideros angustifolia*. We also wanted to examine whether in-situ water availability affected N source or uptake in the invasive species. We found *A. mearnsii* was indeed functioning differently from non-N-fixing native species, and had considerably higher foliar %N. Interestingly, ^{15}N abundance and uptake were associated with site hydrology, meaning water availability should be scrutinised when assuming N-fixing in *A. mearnsii* using δ^{15}N. Nonetheless, higher water availability to *A. mearnsii* in fynbos riparian ecosystems did increase foliar N uptake. This has implications for prioritizing clearing of sites with increased nutrient deposition, such as dense stands in relatively moist riparian zones.

Keywords: δ^{15}N, *Acacia mearnsii*, Black Wattle, South Africa, stable isotopes, water stress

INTRODUCTION

The invasion of non-native *Acacia* into South African landscapes threatens the future integrity of many ecosystems (Le Maitre et al., 2011), especially those of the hyper-diverse fynbos biome (Wilson et al., 2014). The extensive invasion of *A. mearnsii* into fynbos riparian ecosystems is of particular concern (Van Wilgen et al., 2012): *A. mearnsii* populations decrease watershed yield of fresh water (Enright, 2000; Dye and Jarmain, 2004; Van Wilgen et al., 2007), and this in a region already predicted to experience more drought events due to climate changes (New, 2002; Steynor et al., 2009). Among the main invasive tree species in South Africa today, *A. mearnsii* is considered the largest contributor to water flow reductions (Le Maitre et al., 2016).

In addition to worsening water stress for native plant communities, nitrogen (N)-fixing *A. mearnsii* will likely affect N cycling and thus local ecosystem functioning (Lee et al., 2017). Non-legumes rely mainly on soil-available ammonium and nitrate, whereas legumes are capable of fixing abundant atmospheric N via microbial symbioses, thereby adding N to the plant–soil system. Legumes are, therefore, expected to have higher levels of N accumulated in plant material compared to reference non-fixers (Wright et al., 2004). For example, in Australia, *A. mearnsii* had significantly higher foliar %N than a co-occurring but non-N-fixer, *Eucalyptus globulus* (Forrester et al., 2007). In nutrient-poor fynbos soils, a higher leaf %N may thus improve growth and persistence of *A. mearnsii* in the landscape (Sardans et al., 2017). Subsequently dense stands of N-enriched *A. mearnsii* would also increase terrestrial and aquatic nutrient pollution in catchments (Chamier et al., 2012; Tye and Drake, 2012).

Nitrogen dynamics in landscapes where legumes invade may vary depending on the relative access of the plant to water in that landscape (Dudley et al., 2014). This N variation might be reflected in site-specific plant performance and thus affect future environmental impact estimations. Since legumes could also acquire different N sources compared to co-occurring native plants (Högberg, 1997; Robinson, 2001), studying the natural abundances of ^{15}N:^{14}N in foliage could further explain variation in N-source uptake between invasive N-fixers and native non-N-fixing plants in the landscape (Nielsen et al., 2016). Interestingly, δ^{15}N appears negatively related to water availability when measured as mean annual precipitation, but positively related to landscape-scale measures such as moisture patches (Handley et al., 1994). Both foliar %N and δ^{15}N could therefore provide an integrated view of how legume invasion has the potential to impact nutrient dynamics in the landscape (Stock et al., 1995), and how these impacts might vary with local water availability (Handley et al., 1994).

Leaf N dynamics of invading *A. mearnsii* under conditions of varying water availability in fynbos riparian zones have not been examined. Higher leaf-N content may lead to greater rates of CO_2 uptake, resulting in a sustained cycle of increased N assimilation in the roots and other organs (Field et al., 1983; Cernusak et al., 2009). By reanalysing published plant water-stress data (δ^{13}C, predawn [$\Psi_{(pd)}$] and midday [$\Psi_{(md)}$] water potentials; Crous et al., 2012) and % foliar N, δ^{15}N, C, and C:N ratios from the same samples, we aimed to study leaf N dynamics as functions of both species-specific functional traits and riparian-specific water availability (*sensu* Schulze et al., 1991). We thus assessed this question of functional variation in a multivariate fashion (Walker et al., 1999; Petchey and Gaston,

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* To whom all correspondence should be addressed.
e-mail: casper@saeon.ac.za

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2002), hypothesising first that the invasive legume would be functionally different from co-occurring native riparian species that are not leguminous (Lee et al., 2017), and second that in-situ water availability would positively correlate with foliar N and δ^{15}N values, potentially affecting N source and uptake by *A. mearnsii* in moister or drier riparian zones.

METHODS

Foundation and extant data

Sites

The evergreen non-native and invasive legume, *Acacia mearnsii* De Wild (Fabaceae), was compared to two native, evergreen, and non-leguminous fynbos riparian species, *Brabejum stellatifolium* (L.) (Proteaceae) and *Metrosideros angustifolia* (L.) (Myrtaceae). These native species were selected as they consistently co-occur with the invasive in the landscape, and are also considered key species in fynbos riparian zones (Galatowitsch and Richardson, 2005). There were no dominant native leguminous tree species for comparison with *A. mearnsii*. The three study sites were the Eerste River at Jonkershoek (33°57.621′S, 18°55.037′E; 200 m a.s.l.), Wit River at Bainskloof (33°34.217′S, 19°08.452′E; 279 m amsl) and Molenaars River at DuToitskloof (33°41.778′S, 19°13.263′E; 297 m amsl). At each site 5 individuals per species were marked for leaf sampling and water-stress measurements ($n = 5$). However, one *A. mearnsii* individual was lost at Jonkershoek during the course of the study due to wood harvesting, rendering $n = 4$ for this site. All sampled tree individuals were between 2 and 4 m in height, and were similar in leaf size.

Foliar carbon and nitrogen stable isotope analysis

Ten mature leaves from the well-exposed outer canopy of each tree were harvested from each individual, stored in paper bags and dried at 40°C until a constant weight was achieved. Dried leaves were crushed into a fine powder with a mortar and pestle and powdered leaf samples of approx. 2.852 ± 0. 025 mg each were analysed for %N, %C, δ^{15}N, δ^{13}C and C:N by combustion in an automated Flash EA 1112 elemental analyser (Carlo-Erba), coupled with a Delta Plus XP isotope mass spectrometer. These analyses were carried out in the Stable Light Isotope Unit, in the Department of Archaeology at the University of Cape Town. Stable isotope data were expressed as:

$$\delta(\mathrm{x})\ (‰) = ((R_{\mathrm{sample}} - R_{\mathrm{standard}}) / R_{\mathrm{standard}}) \times 1\,000 \qquad (1)$$

where R_{sample} is the ^{13}C/^{12}C or ^{15}N/^{14}N ratio of the sample.

Leaf water potentials

Water potential measurements were carried out on the same individuals. Three healthy shoots were cut from each individual and leaf water potential was measured immediately on site using a pressure chamber (PMS Instruments, Covallis, Oregon, USA). Predawn and midday water potential measurement were replicated on three non-consecutive days for each species across all sites, ultimately totalling 45 predawn and 45 midday measurements for each species at each site. Sampling took place in the summer season (Dec–Feb 2008/2009), when most plants would experience the most water-stressed conditions. Leaf water potential data were previously published in Crous et al. (2012).

Explanatory variables

The following categorical explanatory variables were included in the multivariate analysis to determine functional differences in foliar %N, %C, δ^{15}N, δ^{13}C and C:N across the three species:

1. **Site water availability:** Sites were categorized by their relative water availability to plants. This was done using predawn water potential values ($\Psi_{(\mathrm{pd})}$); which correspond to the maximum level of hydration and the soil moisture that the plants have access to), midday values ($\Psi_{(\mathrm{md})}$); which provide the water potential at the maximum level of water deficit for the day), and site-specific geomorphological conditions (which may influence water availability at each site) as described in Crous et al. (2012). These data suggested Bainskloof to be the riparian zone where trees experienced the most water stress (lowest water potentials), even though this catchment does not have the lowest streamflow volume. Trees at the other two sites had similar, higher water potentials during prevailing summer conditions. Trees at Bainskloof ($n = 15$) were thus categorized as having lower site water availability with those at Jonkershoek and DuToitskloof having higher site water availability ($n = 29$).
2. **Relative root access to groundwater:** The relative root access to groundwater during the day ($\Psi_{(\mathrm{delta})} = \Psi_{(\mathrm{md})} - \Psi_{(\mathrm{pd})}$) provides an indication of the range of water potentials a species operates at during transpiration. A lower $\Psi_{(\mathrm{delta})}$ could indicate a species restricting transpiration via stomatal closure, and a higher value could indicate a species transpiring to a greater degree, or that resistance to water transport is greater in the soil–plant hydraulic pathway.
3. **Plant functional type:** Plant functional type was categorized as being a legume or not, which indicates the potential for N fixation and greater N-uptake capacity in nutrient-limited environments.

Statistical analyses

To test for significant differences in plant function between the invasive legume and the native non-legumes, permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed in PRIMER 6 (PRIMER-E 2008, Lutton, UK). Multivariate dissimilarity was calculated using Euclidean distances, and using 9 999 permutations (Anderson 2001). Then, to test which of site water availability, relative root access to groundwater [$\Psi_{(\mathrm{delta})}$], and plant functional type more strongly explained plant functional differences (%N, %C, δ^{15}N, δ^{13}C and C:N), and if these explanatory variables were positively or negatively related to traits, we executed a redundancy analysis (RDA) (Lepš and Šmilauer, 2003). The significances of the overall RDA ordination as well as the first canonical axis were first calculated using 9 999 permutations. As these tests were significant at the 95% level, we proceeded with forward selection of the explanatory variables to rank the most important ones in this dataset. Explanatory variables that were linearly dependent were excluded from the analysis, but their position in ordination space (the plot) kept as they remain useful to compare to other treatments. All response variables were centred and standardized because of varying measurement units. The same RDA procedure was used to assess the species-specific responses in %N, %C, δ^{15}N, δ^{13}C and C:N to site water availability or relative root access to groundwater. All these analyses were calculated using CANOCO 5.

RESULTS

Comparing foliar %N, %C, δ^{15}N, δ^{13}C and C:N revealed significant dissimilarity between the invasive legume *A. mearnsii* and two co-occurring non-legume native species (PERMANOVA, t = 13.29, P<0.001; data not shown). The analysis showed two significant explanatory axes in this dataset (Fig. 1). Whereas plant functional type, site water availability, and relative root access to groundwater [$\Psi_{(delta)}$] together explained 70.2% of the constrained variation in foliar N, C, δ^{15}N, δ^{13}C and C:N (RDA, Test on All Axes: *Pseudo-F* = 34.1, *P* < 0.001; data not shown), after forward selection, only plant functional type (*Pseudo-F* = 45.6, *P* < 0.001) and site water availability (*Pseudo-F* = 34.1, *P* < 0.001) were included in the final ordination (Fig. 1). Together, these two variables explained 68% of the constrained variation, with site water availability explaining 16% of the variation, particularly variation in leaf δ^{15}N and δ^{13}C, and functional type the rest (52% variation explained). Specifically, %C, %N and C:N levels are directly associated with being leguminous, but δ^{15}N and δ^{13}C are less species-specific and more related to local moisture conditions.

Testing the effects of the water access on leaf N, C, δ^{15}N, δ^{13}C and C:N within species revealed that, for both *A. mearnsii* and *M. angustifolia*, site water availability was most significant in structuring trait variation (variance explained = 56.9%, *Pseudo-F* 15.8, *P* < 0.001; and variance explained = 38.7%, *Pseudo-F* 8.2, *P* < 0.001, respectively; Fig. 2). For *B. stellatifolium* the relative root access to groundwater ($\Psi_{(delta)}$) was the strongest predictor of changes in leaf N, C, δ^{15}N, δ^{13}C and C:N (variance explained = 23.7%, *Pseudo-F* 4.0, *P* = 0.007; Fig. 2). For all species, total foliar %N, and fractionation resulting in ^{15}N enrichment, was significantly associated with experiencing less water stress.

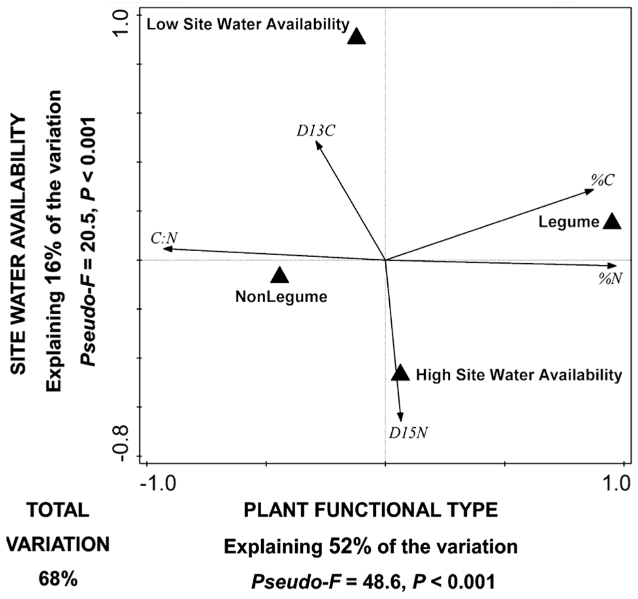

Figure 1

Redundancy analysis (RDA) showing the strength of association and variation explained by plant functional type (legume/non-legume) and site water availability on foliar N, C, δ^{15}N, δ^{13}C and C:N.

DISCUSSION

Compared with two co-occurring native non-legumes, the invasive legume *A. mearnsii* functioned differently in fynbos riparian zones. *Acacia mearnsii* had higher leaf %N than the non-leguminous natives as well as higher leaf %C and a lower C:N ratio. In contrast, δ^{15}N levels for all species

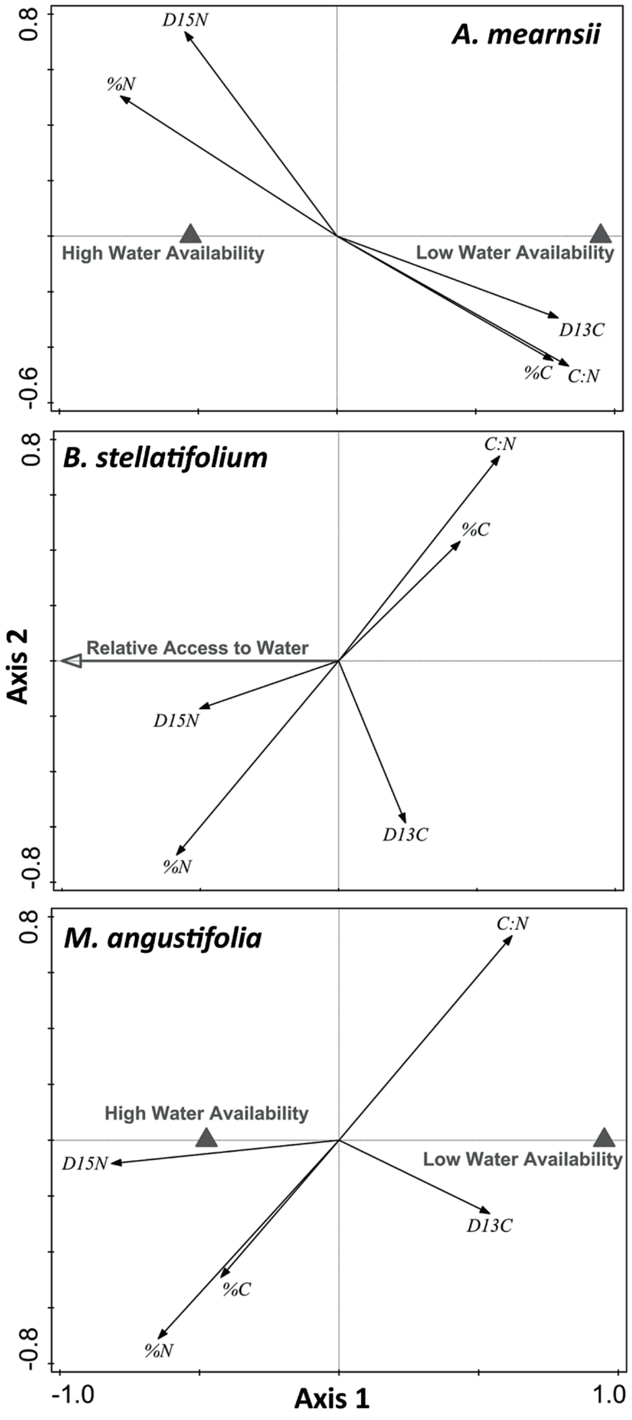

Figure 2

Species-specific redundancy analyses (RDAs) in order to find the strongest explanatory variable(s) (site water availability and relative root access to water [$\Psi_{(delta)}$]) shaping the distribution in foliar %N, %C, δ^{15}N, δ^{13}C and C:N within each species.

grouped orthogonal to plant functional type, as did δ^{13}C. This suggests that water availability was influencing foliar ^{15}N abundance independently from the ability of *A. mearnsii* to fix atmospheric N. The importance of local water availability on plant N dynamics for the invasive legume was further highlighted as water stress decreased the amount of N in the leaf tissue. Consistent with Funk et al. (2017), in-situ water availability, as opposed to broad-scale water availability, would aid predictions on the functioning and ecosystem impact of N-enriched *A. mearnsii* in fynbos riparian ecosystems.

There was a positive relationship between foliar δ^{15}N within these riparian zones and in-situ water availability. For all species examined here, higher water availability in the riparian zone was associated with ^{15}N enrichment. At the regional scale, mean annual precipitation as an indicator of water availability negatively correlated with δ^{15}N when comparing southern African C_3 species (Swap et al., 2004). However, when plants were compared among sites with similar precipitation patterns but different site hydraulic characteristics, for example, landscape topography and moist patches, then local-scale water availability was positively correlated with δ^{15}N (Handley et al., 1999; Ruiz-Navarro et al., 2016). Leaf δ^{15}N in the examined riparian ecosystems appears less related to being leguminous than foliar %N, %C and C:N. We conclude that the patterns in N isotope enrichment described here were likely associated with local-scale geomorphological and other catchment-specific hydraulic characteristics (Handley et al., 1999; Averill and Finzi, 2011), as was also evident for δ^{13}C in these riparian zones (Crous et al., 2012).

Species-specific symbioses with microbes also contribute to variation in foliar δ^{15}N (Högberg, 1997; Boddey et al., 2000; Robinson, 2001; Craine et al., 2009). However, the role of N fixation by mycorrhizal fungi or bacterial symbionts in altering the N source available to *A. mearnsii* is unclear from our data. The three co-occurring species varied considerably in δ^{15}N along the same axis making it difficult to infer the specific level of N fixed by the legume from the atmosphere (Högberg, 1997; Boddey et al., 2000). Instead, the site-moisture association found in this study suggests that nitrification could have been greater under higher site water availability, leaching ^{15}N-depleted NO_3^- from the soil, and leaving more ^{15}N enriched NH_4^+ (Högberg, 1997). Still, the microbes suitable for forming nodules are present for *A. mearnsii* species in South Africa (Joubert, 2003; see also Stock et al., 1995). The contribution of symbiotic N-fixation to leaf δ^{15}N variation needs further investigation in relation to a local water availability gradient.

Implications for fynbos riparian zone management

Persisting populations of *A. mearnsii* in fynbos riparian ecosystems could severely affect N cycling in the long run, accentuating the effects of non-native legume introductions into historically N-poor soils (Musil and Midgley, 1990; Lee et al., 2017). For example, all the sampled *A. mearnsii* leaves had lower C:N ratios than the sampled native species. Since *A. mearnsii* forms dense stands and replaces mostly non-leguminous native trees in fynbos riparian zones, the quality of leaf litter inputs to streams will change dramatically with invasion, an impact now widely anticipated (Witkowski, 1991; Stock et al., 1995; Forrester et al., 2007; Chamier et al., 2012; Dudley et al., 2014; Lee et al., 2017). Considering water run-off in especially steep, mountainous catchments (such as our studied catchments), this extra N contributed by invasive plants could be detrimental for water quality due to eutrophication (Vitousek et al., 1997; Drake, 2011; Chamier et al., 2012; Tye and Drake, 2012). In addition, many fynbos riparian areas are already flowing through agricultural regions, a feature often associated with increased nitrogen deposition into the environment (Carpenter et al., 1998; Drake, 2011). Ongoing sewage dumping into rivers is also likely to further exacerbate this situation.

The primary way South Africa addresses *A. mearnsii* invasion is through clearing, but this is often time consuming, expensive, and only done in a fraction of the total invaded area. Because trait expression of the invasive legume varied according to site water availability, indicating that in-situ functioning and impacts of *A. mearnsii* vary across riparian zones, we can potentially use these data to help prioritize the clearing of wetter sites, particularly if nutrient deposition is the main concern (Emmett et al., 1998). This would complement national clearing initiatives already in place such as South Africa's 'Working for Water' programme (Van Wilgen et al., 1998), where funding is limited (Van Wilgen et al., 2012).

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APPENDIX

TABLE A1
Raw foliar N, C, δ^{15}N, δ^{13}C and C:N data measured for the invasive legume *Acacia mearnsii*, and two native co-occurring non-legumes *Brabejum stellatifolium* and *Metrosideros angustifolia* in three fynbos riparian zones, South Africa.

Species	Individual	D15N	Foliar %C	Foliar %N	C:N	D13C
JONKERSHOEK						
B. stellatifolium	1	0.60	49.19	0.94	52.31	–26.04
B. stellatifolium	2	0.42	49.43	0.98	50.30	–26.79
B. stellatifolium	3	1.55	48.82	0.94	51.84	–24.17
B. stellatifolium	4	1.43	49.38	1.21	40.78	–25.38
B. stellatifolium	5	1.41	47.52	1.15	41.14	–24.12
Mean		*1.08*	*48.87*	*1.05*	*47.27*	*–25.30*
M. angustifolia	1	1.10	48.68	1.22	39.99	–23.31
M. angustifolia	2	0.39	48.78	0.91	53.72	–23.67
M. angustifolia	3	–1.86	48.96	1.38	35.47	–25.04
M. angustifolia	4	–1.17	49.15	1.30	37.94	–23.13
M. angustifolia	5	1.86	48.76	1.24	39.42	–22.64
Mean		*0.06*	*48.87*	*1.21*	*41.31*	*–23.56*
A. mearnsii	1	–2.30	54.26	2.82	19.27	–25.93
A. mearnsii	2	–2.13	53.24	3.06	17.40	–25.71
A. mearnsii	3	–2.11	52.26	2.97	17.59	–25.39
A. mearnsii	4	–1.83	52.01	3.44	15.13	–24.72
Mean		*–2.09*	*52.94*	*3.07*	*17.35*	*–25.44*
BAINSKLOOF						
B. stellatifolium	1	–4.92	49.83	0.92	53.96	–25.22
B. stellatifolium	2	–2.71	49.17	0.96	51.26	–24.45
B. stellatifolium	3	–3.31	49.70	0.95	52.20	–24.71
B. stellatifolium	4	–2.42	48.61	1.02	47.63	–24.30
B. stellatifolium	5	–2.10	48.43	1.02	47.66	–25.21
Mean		*–3.09*	*49.15*	*0.97*	*50.54*	*–24.78*
M. angustifolia	1	–4.66	49.62	0.99	50.26	–21.14
M. angustifolia	2	–6.52	48.13	0.92	52.25	–21.85
M. angustifolia	3	–3.10	48.33	1.00	48.41	–22.37
M. angustifolia	4	–3.23	48.70	0.96	50.96	–23.94
M. angustifolia	5	–5.14	48.49	0.83	58.26	–24.19
Mean		*-4.53*	*48.65*	*0.94*	*52.03*	*–22.70*
A. mearnsii	1	–1.17	55.38	2.67	20.74	–24.22
A. mearnsii	2	–3.06	55.52	2.10	26.38	–24.32
A. mearnsii	3	–1.91	54.61	2.62	20.87	–22.70
A. mearnsii	4	–3.23	54.46	2.25	24.24	–23.38
A. mearnsii	5	–2.57	53.72	2.34	22.99	–24.31
Mean		*–2.39*	*54.74*	*2.39*	*23.04*	*–23.79*

TABLE A1 (cont.)
Raw foliar N, C, δ^{15}N, δ^{13}C and C:N data measured for the invasive legume *Acacia mearnsii*, and two native co-occurring non-legumes *Brabejum stellatifolium* and *Metrosideros angustifolia* in three fynbos riparian zones, South Africa.

Species	Individual	D15N	Foliar %C	Foliar %N	C:N	D13C
DUTOITSKLOOF						
B. stellatifolium	1	−2.07	49.30	0.98	50.37	−27.73
B. stellatifolium	2	−1.96	47.69	0.88	54.43	−27.45
B. stellatifolium	3	−2.17	48.72	1.13	43.15	−25.45
B. stellatifolium	4	−2.36	48.14	1.22	39.58	−27.48
B. stellatifolium	5	−1.82	49.86	0.81	61.54	−26.04
Mean		*−2.08*	*48.74*	*1.00*	*49.81*	*−26.83*
M. angustifolia	1	−2.64	49.81	1.18	42.131	−25.60
M. angustifolia	2	−1.99	48.79	0.87	56.256	−25.23
M. angustifolia	3	−0.11	49.27	1.15	42.753	−23.82
M. angustifolia	4	−0.28	50.07	1.29	38.844	−23.88
M. angustifolia	5	−0.95	48.95	1.29	38.086	−26.24
Mean		*−1.19*	*49.38*	*1.16*	*43.614*	*−24.95*
A. mearnsii	1	−0.57	50.59	3.71	13.637	−27.88
A. mearnsii	2	−1.43	52.71	2.81	18.77	−26.15
A. mearnsii	3	−0.46	51.76	3.02	17.111	−27.05
A. mearnsii	4	−1.24	53.13	2.92	18.164	−26.11
A. mearnsii	5	−0.82	52.21	2.94	17.78	−28.03
Mean		*−0.90*	*52.08*	*3.08*	*17.092*	*−27.05*