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Effect of different nitrogen fertilizer application rates on *Dichrostachys cinerea* and *Acacia karroo* sapling growth, foliar nutrient and antinutrient concentrations in a southern African savanna

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Abstract

Nitrogen (N) fertilization influences woody plant growth, foliar nutrient and antinutrient concentrations. We conducted a nursery experiment to determine the effect of five N fertilization rates on *Dichrostachys cinerea* and *Acacia karroo* sapling height, basal diameter, growth of longest shoots and root length, crude protein (CP), acid and neutral detergent fiber (NDF), condensed tannins (CT) and total phenolic (TP) concentrations. Eight potted plants of each woody species were allocated to each of five N fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N/kg of soil) in a completely randomized design. Different N fertilization rates did not result in varying plant growth in terms of height, stem diameter and root length in both *D. cinerea* and *A. karroo*. However, N fertilization rate of 0.63 g N per kg of soil resulted in an increase in length of the longest shoot in both *D. cinerea* and *A. karroo*. Foliar CP increased with an increase in N fertilization rate up to 0.63 g N per kg of soil, while acid and NDF, CT and TP were lowest at N fertilization rates of 0.63 g N per kg of soil in both *D. cinerea* and *A. karroo*. We conclude that N did not limit plant growth, and therefore N fertilization is unlikely to significantly improve sapling growth. However, N fertilization improved foliar CP and reduced CT and TP concentrations in both *D. cinerea* and *A. karroo* improving their nutritive value to browsers.

KEYWORDS
carbon-based plant secondary metabolites, crude protein, fiber, nitrogen-fixing, plant height

1 | INTRODUCTION

In natural ecosystems soil nutrients are supplied through decomposition, weathering of rocks, atmospheric deposition and biological fixation in the case of nitrogen (N) (Wright et al., 2011). Woody plant growth in natural ecosystems is often limited by soil nutrient availability, particularly N (Goergen, Chambers, & Blank, 2009). A positive response to experimental fertilization can be used to determine if a nutrient is limiting plant growth (Vitousek, Porder, Houlton, & Chadwick, 2010). For example, a strong positive response to N fertilization indicates that N is limiting plant growth (LeBauer & Treseder, 2008). Few experimental studies have been conducted to determine how different fertilizer levels influence woody plant growth in African savanna ecosystems. Woody plant species such as *Dichrostachys cinerea* (L.). Wright and Arn and *Acacia karroo* Hayne can fix N symbiotically improving soil N in natural ecosystems (Ludwig, De Kroon, Berendse, & Prins, 2004). To better manage savanna ecosystems a clear understanding of how N-fixing trees respond to varying levels of soil N is important.

Rapid sapling growth is important for woody plants to quickly grow beyond the browsing height of herbivores (Vadigi & Ward, 2012) and escape the "flame zone" (Lawes, Adie, Russell-Smith, Murphy, & Midgley, 2011; Vadigi & Ward, 2013). Thus, woody plants need to invest in rapid sapling growth as poor growth limits their recruitment into the tree strata (Midgley & Bond, 2001). Soil nutrients are important for sapling growth (Kraaij & Ward, 2006). For instance, Wakeling, Cramer, and Bond (2010) found *Acacia* species sapling growth to be strongly influenced by soil nutrients availability. Plant growth in savanna ecosystems is often limited by N shortages (Bremen & de Wit, 1983), with N fertilization associated with an increase in tree growth (Santiago et al., 2012; Scogings, 2018; Wright et al., 2018). *D. cinerea* and A*cacia* species, such as *A. karroo* are N-fixing trees whose growth should not be limited by shortages of N (Ward, 2005). Interestingly, studies on *Acacia* species at different sites showed mixed tree responses to N fertilization. For example, Gowda, Albrectsen, Ball, Sjoberg, and Palo (2003) reported a significant increase in the mass of shoots, leaves, and spines of *Acacia tortilis* with increasing fertilizer addition. Kambatuku, Cramer, and Ward (2013) reported varying shoot elongation of *Apis mellifera* seedlings with different fertilizer amounts. However, Otuba and Weih (2015) reported N fertilization as having no effect on the growth of *Acacia senegal* and *Acacia sieberiana*.

Applying fertilizer containing growth limiting nutrients to woody plants has been found to increase their foliar nutrient concentration, while reducing carbon-based secondary plant metabolites (Bryant, Chapin III, & Klein, 1983), which improves their acceptability, to herbivores (Holechek, Pieper, & Herbel, 2001). For instance, N fertilization is associated with an increase in foliar crude protein (CP) content (Bryant, Clausen, Reichardt, McCarthy, & Werner, 1987; Wright et al., 2018), a decline in condensed tannin (CT) concentrations (Bryant et al., 1987; De Long, Sundqvist, Gundale, Giesler, & Wardle, 2016), and total phenolics (TP) concentrations (Kainulainen, Holopainen, Palomaki, & Holopainen, 1996). We could not find other studies that have previously investigated the effects of N fertilization on leaf chemical traits for N-fixing African savanna tree species.

We conducted experiments in a nursery to evaluate the effects of different N fertilizer application rates on *D. cinerea* and *A. karroo* sapling growth in terms of plant height, basal diameter, length of longest shoots, and root length in a south African savanna. In addition, we assessed the effect of N fertilization on leaf nutrient (CP) and antinutrient concentrations (neutral and acid detergent fiber [ADF], CT and TP). *D. cinerea* and *A. karroo* were selected for the study because of their abundance and role as browse species for both domestic and wild herbivores. We tested the hypotheses that an increase in N fertilizer application rates will (a) not increase plant growth in terms of height, basal diameter, length of the longest shoot, and root length; (b) increase foliar CP content; and (c) reduce CT, TP concentrations, and fiber (both neutral and ADF) content of *D. cinerea* and *A. karroo* saplings.

2 | METHODS

2.1 | Study site

The study was carried out at the Midlands State University (MSU) nursery (19° 30′ 53.6″ S; 29° 50′ 17.3″ E). The area receives an average annual rainfall of 684 mm in the summer season (October–April). The mean annual temperature is 22.6°C and the area is 1,428 m a.s.l.

2.2 | Study species

The species selected for this study were *D. cinerea* (L.). Wright and Arn. and *A. karroo* Hayne, two of many fine-leafed N-fixing tree species in African savannas. Fine-leafed woody species have higher leaf nutrient concentrations and are important browse species for both domestic and wildlife browsers (Wigley, Fritz, & Coetsee, 2018). *D. cinerea* and *A. karroo* are widely distributed and abundant in southern Africa (Nyamukanza & Scogings, 2008). *D. cinerea* is a semideciduous to deciduous shrub or small tree which occurs in a wide range of natural habitats and grows to a height of up to 7 m tall (Orwa, Mutua, Kindt, Jamnadass, & Anthony, 2009). It is spinescent with spines which grow from modified side shoots and has strong alternate thorns which are up to 8 cm long and almost at right angles, slightly curved, grow out of the branches, and may bear leaves at the base. *A. karroo* is a small to medium-sized, fast-growing thorny tree, but can grow to a height of 12 m (Brown, N'gambi, & Norris, 2016). It has long, straight, whitish thorns (2–5 cm). *A. karroo* is adapted to areas of low and moderate rainfall (Pooley, 1998), and can tolerate severe and frequent defoliation (Teague, 1988).

2.3 | Sapling production

Seeds of *D. cinerea* and *A. karroo* were collected from the communal rangelands next to MSU nursery and scarified by soaking in concentrated sulfuric acid for 1 hr. They were then washed thoroughly with water to remove the traces of acid and then soaked in water for 12 hr. The seeds were then sown in plastic pots (0.25 m diameter × 0.25 m deep) with each pot containing 12 kg of soil ($pH_{(CaCl2)}$—5.9; N—11 ppm; P—90 ppm; K—0.20 meq/100 g; Ca—7.22 meq/100 g; Mg—3.63 meq/100 g) (Nyamukanza unpublished data) obtained from the communal rangelands next to MSU where seeds of *D. cinerea* and *A. karroo* were collected. Soil N concentration was determined using the Kjeldahl method. The seedlings were raised under natural light and temperature in the nursery under a black shade cloth which allowed 60 % natural lighting to pass through for 4 months and were watered once every 2–3 days and weeded.

2.4 | Treatments

After 4 months, each pot was randomly assigned to one of five N fertilization rate treatments viz. 0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil in a completely randomized design. Ammonium nitrate, a product of Windmill™, containing 34.5% N was used as a source of N. Fertilizer granules were applied on top soil in the pots after watering, once as per treatment. Eight plants from each woody species were allocated to each treatment. The following parameters were measured: (a) plant height using a 2 m metal rod from the top of the pot to the top of the plant, (b) basal diameter using Vernier calipers at 5 cm aboveground level and (c) length of side shoots, using a tape measure by taking the length of the longest shoot, before allocation to treatments and the measurements were used to monitor the growth of the saplings.

After a further 4 months the following parameters were measured: (a) plant height, (b) basal diameter, (c) root length, and (d) length of longest shoot. The saplings were then harvested, separated into morphological parts (leaves, stems and roots) and dried in an oven at 60°C for 48 hr. Each plant part was weighed and leaf samples were milled to pass through a 1.0 mm screen using a Wiley Mill.

2.5 | Leaf chemical analysis

Leaf samples were analyzed for CP, fiber (acid and neutral detergent fiber [NDF]), TP and CT concentration. Part of leaf sample was digested with 20 ml sulfuric acid at 350°C for 6 hr using the Kjeldahl method. N concentration in the digest was then determined by colorimetry (Association of Official Analytical Chemists, 2012). CP concentration was estimated as N × 6.25. NDF and ADF were determined according to Van Soest, Robertson, and Lewis (1991) using the ANKOM Technology Technique. NDF consists of hemicellulose, cellulose, cutin and lignin while ADF consists of cellulose, cutin and lignin. Hemicellulose and cellulose are both structural carbohydrates that can be digested to some extent by rumen bacteria to provide energy. Lignin is an indigestible compound that reduces the digestibility of cellulose and hemicellulose by rumen microbes. Both NDF and ADF are measures of plant fiber content. NDF was determined by weighing 1 g of ground sample (1 mm screen) and placing in conical flask, adding 100 ml neutral detergent solution, 2 ml decahydronaphthalene and 0.5 g sodium sulfite. Then heating to boil and refluxing for 60 min. Pouring into Buchner flask and filtering and then rinsing with hot water and acetone and drying at 105°C to constant weight. ADF was determined by weighing 1 g of sample into a reflux flask, adding 100 ml of cold acid detergent solution and 2 ml decahydronaphtalene, heating to boiling and refluxing for 60 min. A residue was obtained by filtering, washing with hot water and drying at 105°C to constant weight.

Two grams of ground leaf samples were mixed with 10 ml of boiling distilled water and then vortexed using a Heidolph REAX 2000 Model 54113 (Heidolph©) for 1 min and sonicated using a Model 90S (Westwood Ultrasonics Ltd) sonicator for 10 min (Bhebhe, Chipurura, & Muchuweti, 2015). The supernatant was used for CT and TP analysis. CT were determined by pouring sample extract (0.5 ml) into test tubes followed by 3 ml of butanol: HCl (95:5 vol/vol) reagent and then 0.1 ml of ferric reagent (2% of ferric ammonium sulfate dissolved in 2 N HCl) (Bhebhe et al., 2015). The test tubes were vortexed, covered with a glass marble and heated in a water bath at 90–100°C for 1 hr (Makkar & Goodchild, 1996), cooled and absorbances read at 550 nm on a spectrometer (Bhebhe et al., 2015). Tannin content was calculated as leucocyanidin equivalent (LE) according to the formula: $c = [A_{550}\ nm/\varepsilon b] \times$ dilution factor, where A_{550}nm is absorbance at 550 nm, c is concentration in (g LE/100 g),ε is 460 (extinction coefficient) and b is 1 cm (path length of light) (Bhebhe et al., 2015; Makkar & Goodchild, 1996). TP content was determined using theFolin–Ciocalteau method with modifications (Bhebhe et al., 2015; Makkar & Goodchild, 1996). Leaf sample extract (50 µl) was mixed with distilled water (950 µl) to make up to 1 ml. Sodium carbonate (2%) (2.5 ml) was then added followed by 500 µl of Folin–Ciocalteau reagent (0.62 g/ml) (Bhebhe et al., 2015). The mixture was incubated for 40 min in a dark place and absorbance read at 725 nm using a spectrophotometer (Bhebhe et al., 2015). Gallic acid (0.5 mg/ml) was used as a standard and the TP concentration was expressed as gallic acid equivalents.

2.6 | Statistical analysis

The effect of N fertilization rate on plant height, basal diameter, length of longest shoot, root length, CP, ADF, NDF, TP and CT were compared using one-way analysis of variance using IBM SPSS v.19 (IBM Corp, IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY). All data were tested for normality using Kolmogorov–Smirnov test and for homogeneity of variance using Levene's test. Significant differences between means were determined using Tukey's post hoc test. The significance level was set at alpha = .05.

3 | RESULTS

Nitrogen fertilization had no significant effect on plant height (*D. cinerea*: $F_{4,35} = 1.34$; $p = .27$; *A. karroo*, $F_{4,35} = 0.65$, $p = .63$; Figure 1), basal diameter (*D. cinerea*, $F_{4,35} = 1.91$, $p = .13$; *A. karroo*, $F_{4,35} = 1.69$, $p = .18$; Figure 2;) and root length (*D. cinerea*, $F_{4,35} = 0.82$, $p = .52$; *A. karroo*, $F_{4.35} = 1.42$, $p = .25$; Figure 3;).

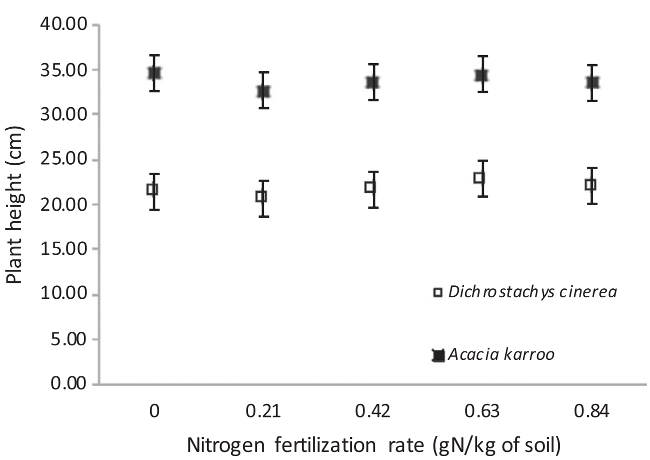

FIGURE 1 Mean (±*SE*) ($n = 8$) plant height of *Dichrostachys cinerea* and *Acacia karroo* saplings under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil). Error bars represent *SE*

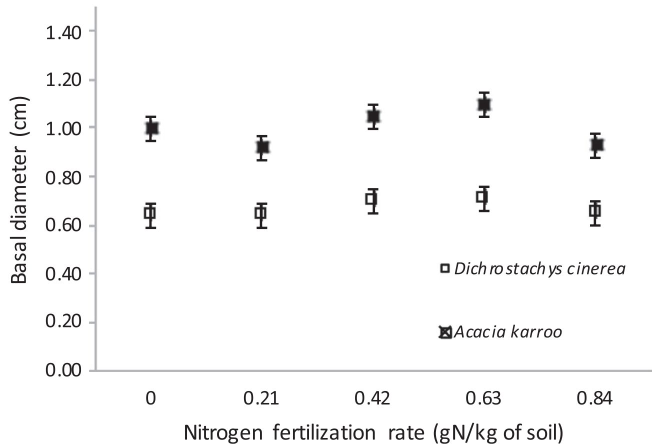

FIGURE 2 Mean (±*SE*) ($n = 8$) basal diameter of *Dichrostachys cinerea* and *Acacia karroo* saplings under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil). Error bars represent *SE*

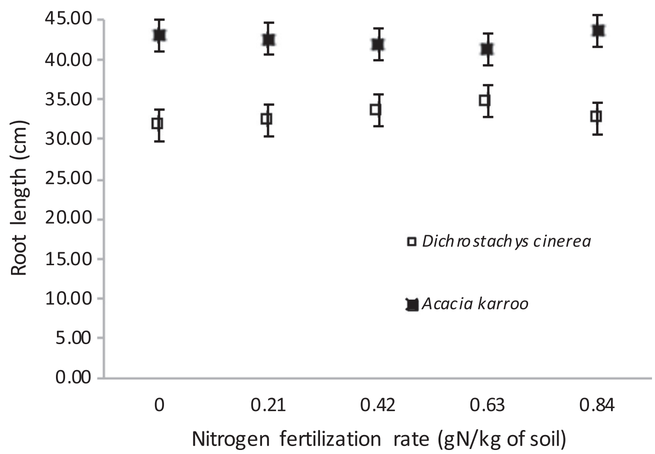

FIGURE 3 Mean (±*SE*) ($n = 8$) root length of *Dichrostachys cinerea* and *Acacia karroo* saplings under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil). Error bars represent *SE*

Nitrogen fertilization had a significant effect on growth of the longest shoot (*D. cinerea*, $F_{4,35} = 4.08$, $p = .04$; *A. karroo*, $F_{4,35} = 265.32$, $p < .001$; Figure 4). Growth of longest shoot was highest at N fertilization rate of 0.63 g N per kg of soil in both *D. cinerea* and *A. karroo*.

CP content significantly increased, while ADF, NDF, CT and TP decreased with an increase in N fertilization up to 0.63 g N per kg of soil in both *D. cinerea* (except for 0 g N per kg of soil N fertilizer) and *A. karroo* (Tables 1, 2).

4 | DISCUSSION

Our findings showed that different N fertilization rates did not result in increased plant growth in terms of

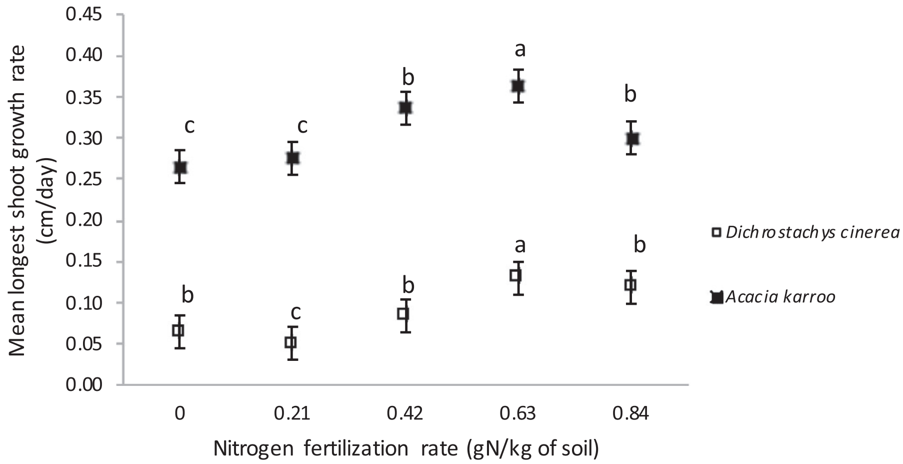

FIGURE 4 Mean (±*SE*) ($n = 8$) longest shoot growth rate of *Dichrostachys cinerea* and *Acacia karroo* saplings under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil). Significant differences ($p < .05$) are denoted by different lowercase letters. Error bars represent *SE*

TABLE 1 Mean (±*SE*) chemical composition (g/kg dry matter) of *Dichrostachys cinerea* leaves under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil)

Nitrogen fertilizer (g N per kg of soil)	Crude protein	Acid detergent fiber	Neutral detergent fiber	Total phenolics	Condensed tannins
0	284.12 ± 0.83^{c}	331.38 ± 0.75 ^{c}	524.00 ± 0.82^{b}	223.62 ± 0.75^{c}	7.28 ± 0.36^{a}
0.21	281.25 ± 0.75^{c}	364.12 ± 0.64^{a}	567.12 ± 0.64^{a}	309.12 ± 0.74^{a}	7.60 ± 0.32^{a}
0.42	296.50 ± 1.40^{b}	342.00 ± 0.53^{b}	525.25 ± 0.59^{b}	249. 25 ± 0.82^{b}	4.61 ± 0.32^{b}
0.63	313.62 ± 0.73^{a}	322.00 ± 0.50^{d}	487.62 ± 0.56^{c}	225.62 ± 0.86^{c}	3.44 ± 0.24^{c}
0.84	281.25 ± 0.75^{c}	344.12 ± 0.44^{b}	526.62 ± 0.84^{b}	209.12 ± 0.64^{d}	3.73 ± 0.12^{c}
$F_{4,35}$ ratios	225.54*	731.14*	1606*	2645*	47.51*

Note: Means with the same letter in the same column are not significantly different ($p < .05$). *$p < .001$.

TABLE 2 Mean (±*SE*) chemical composition (g/kg dry matter) of *Acacia karroo* leaves under five nitrogen fertilization rates (0, 0.21, 0.42, 0.63 and 0.84 g N per kg of soil)

Nitrogen fertilizer (g N per kg of soil)	Crude protein	Acid detergent fiber	Neutral detergent fiber	Total phenolics	Condensed tannins
0	206.88 ± 0.61^{e}	327.12 ± 0.93^{a}	407.12 ± 0.95^{a}	124.25 ± 1.05^{a}	4.50 ± 0.33^{a}
0.21	215.75 ± 0.41^{d}	292.00 ± 0.50^{b}	396.75 ± 0.49^{b}	123.88 ± 0.97^{a}	2.90 ± 0.04^{b}
0.42	246.25 ± 0.94^{c}	277.88 ± 0.30^{c}	376.38 ± 0.68^{c}	109.38 ± 0.75^{b}	2.98 ± 0.16^{b}
0.63	276.00 ± 0.42^{a}	273.12 ± 0.30^{d}	344.88 ± 0.90^{e}	87.13 ± 0.79^{d}	1.83 ± 0.05^{c}
0.84	249.87 ± 0.83^{b}	273.62 ± 0.32^{d}	368.12 ± 0.48^{d}	104.00 ± 0.98^{c}	2.73 ± 0.07^{b}
$F_{4,35}$ ratios	1692*	1850*	1133*	284.27*	33.24*

Note: Means with the same letter in the same column are not significantly different ($p < .05$). *$p < .001$.

height, basal diameter and root length in both *D. cinerea* and *A. karroo*. Mopipi, Trollope, and Scogings (2009) found N fertilization to have no effect on *A. karroo* seedlings growth. Exceptionally, N fertilization rate of 0.63 g N per kg of soil resulted in an increase in length of the longest shoot in both *D. cinerea* and *A. karroo*. Nitrogen fertilization rate of 0.63 g N per kg of soil was optimal for shoot growth. The ability of both *D. cinerea* and *A. karroo* to fix N presumably meant that the plants did not benefit from N fertilizer application (Aubrey & Reynolds, 2002). Vadigi and Ward (2014) reported N fertilization as having mixed effects on woody species stem height and basal diameter growth. For example, N fertilization increased stem growth in *A. tortilis* but not in *A. karroo*, *A. sieberiana* and *Acacia nigrescens*, while basal diameter increased in *A. karroo* and *A. sieberiana* but not in *A. nigrescens* and *A. tortilis*. The lack of plant growth responses to N fertilization could be attributed to the fact that when N availability is high plants become carbon limited (Scogings, Hjalten, & Skarpe, 2011). Carbon is required for the growth of new shoots (Fornara & Du Toit, 2007). In addition, low soil phosphorus levels could have limited the demand for N by the plants resulting in poor growth responses (Kambatuku et al., 2013).

Foliar nutritive value of both *D. cinerea* and *A. karroo* improved with an increase in the amount of N fertilizer applied. For instance, foliar CP increased with an increase in N fertilization rate up to 0.63 g N per kg of soil, while ADF, NDF, CT and TP were lowest at N fertilization rate of 0.63 g N per kg of soil in both *D. cinerea* and *A. karroo*. Nitrogen fertilization rate of 0.63 g N per kg of soil were optimal as the nutritive value was lower at 0.84 g N per kg of soil. High N fertilizer application increases soil mineral salts negatively affecting plant water use. Plant foliar nutrient and antinutrient responses to N fertilization between 0.21 and 0.84 g N per kg of soil point to some form of weak "hump-shaped" curve with a peak at 0.63 g N per kg of soil N fertilizer for CP and an inverse "hump-shaped curve" for ADF, NDF, TP and CT. Scogings et al. (2011) suggested that woody plant species responses to resource (including N) availability tend to assume a hump-shape. Scogings and Mopipi (2008) reported N fertilization as reducing leaf NDF in *A. karroo* saplings. Our results are consistent with the prediction that N fertilization increases the nutritional value of woody plant foliage through an increase in foliar N and a decrease in carbon-based plant secondary metabolites (Bryant et al., 1983, 1987; Kraus, Zasoski, & Dahlgren, 2004; Waring et al., 1985). An increase in demand for carbon (carbon limitation) to meet plant growth requirements results in a decrease in tannins and phenols concentrations (Scogings et al., 2011). Nitrogen fertilization alters the carbon/nutrient balance of woody plants, increasing their nutritive value to herbivores (Bryant et al., 1987). An increase in woody plant nutrients (such as CP) and a decrease in antinutrient concentrations (such as fiber and CT) will improve their acceptability to browsing herbivores (du Toit, Bryant, & Frisby, 1990; Fornara & Du Toit, 2007). Woody plants with high CP and low carbon-based secondary metabolites content are considered to be the least successful at limiting herbivory (Wigley et al., 2018). Thus, N fertilization could be beneficial to herbivores through provision of high-quality browse. Nitrogen fertilization could alter utilization of browse in favor of fertilized plants. Both *D. cinerea* and *A. karroo* have high CP content and are fine-leaved spinescent species that depend on chemical (such as CT) and physical (e.g., spines) defenses against herbivory.

Our study findings imply that in fertile soils, woody plants will produce lower CT and TP concentrations and higher CP content, making them attractive to browsers than would, presumably, occur in infertile soils. In natural rangelands, soil enrichment is through dung and urine deposition by herbivores, with N being the major nutrient deposited (Van Der Waal et al., 2011). In addition, our study showed that native woody plant seedlings can be successfully raised in a nursery before being transplanted into natural ecosystems to restore degraded rangelands and improve availability of browse (Ewing & Best, 2004).

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