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Short communication

Variation in rhizosphere nutrient cycling affects the source of nitrogen acquisition in wild and cultivated *Aspalathus linearis* (N.L.Burm.) R.Dahlgren plants

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ABSTRACT

We investigated the different functional strategies of N nutrition of cultivated and wild varieties of the legume *Aspalathus linearis* (N.L.Burm.) R.Dahlgren during wet and dry seasons in the Cape Floristic Region. The study addressed differences in soil and plant N cycling in cultivated and wild varieties of *A. linearis*. The seasonal variation in biological N_2 fixation (BNF), soil microbial activity, as well as N cycling strategies, were investigated in wild and cultivated varieties of the legume of *A. linearis*. Fresh leaf samples, leaf litter samples and soil samples were collected during dry and wet seasons for both cultivated and wild-growing *A. linearis*. Plant samples were analysed for $\delta^{15}N$ and total N content. Percentage N resorption was calculated. Soil N, C, P, urease and β-glucosidase were determined directly at the rhizosphere and in bulk soil. During the wet seasons, wild plants had an increased reliance on biological N_2 fixation (BNF). This was attributed to an increase in soil microbial activity, which can lead to competition between roots and soil microbes for mineralized soil N. No trade-off between N sources in plants was found and plants tend not to rely more on BNF when microbial activity is low. Wild and cultivated *A. linearis* plants use different N cycling strategies, where wild plants are more reliant on biological N_2 fixation (BNF) whilst cultivated plants tend to retain N.

1. Introduction

Aspalathus linearis is a commercially valuable legume growing in the dry and nutrient-poor Fynbos of the Cape Floristic Region (CRF) (Lötter and Maitre, 2014). With the predicted increase in drought in the CFR, the plant and its symbionts could potentially suffer a loss of function and become less productive. Free-living microbes in the soil that contribute to nutrient cycling could also be influenced by climatic changes, and a decrease in their activity could affect plant productivity (Classen et al., 2015). The establishment of the legume-rhizobia symbiosis can be influenced by various factors, particularly drought stress (Kirda et al., 1989). In legumes, drought stress has a greater effect on Biological N_2 fixation (BNF) than on various other physiological functions (Abdel-Wahab et al., 2002; Wassie et al., 2007; Woldeyohannes et al., 2007; Gil-Quintana et al., 2013; Bargaz et al., 2015). BNF by legumes has high energy costs and requires high levels of phosphorous (Kouas et al., 2009), thus low P-levels can constrain N_2-fixation as it limits plant or nodule growth (Almeida et al., 2000; Weisany et al., 2013).

Free-living soil microbes assist in nutrient cycling by breaking down organic matter to inorganic forms which can be absorbed by plants. The soil microbes secrete extracellular enzymes to degrade complex polymers (Schimel and Bennett, 2004; Beschoren da Costa et al., 2013) and these enzymes are often correlated with nutrient availability (Asmar et al., 1994; Marschner et al., 2004; Lugtenberg and Kamilova, 2009). The activity of these enzymes decreases with a decrease in plant cover (Garcia et al., 2002) and even a slight decrease in soil moisture also decreases enzyme activity (Sardans and Peñuelas, 2005). An increase in drought across years or seasons can thus influence nutrient availability and plant productivity (Pérez-Fermández et al., 2015). Free-living nitrogen-fixing bacteria fix significant amounts of atmospheric nitrogen, which then becomes accessible for acquisition by plants. However, microbes that compete with plants for nutrients can immobilise nitrogen and this can act as short-term nitrogen sink (Zogg et al., 2000).

A. linearis (Fabaceae) is a polymorphic Fynbos species and the

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various wild forms have distinct morphological and geographical characteristics (Joubert et al., 2008; Hawkins et al., 2011). The red cultivated Nortier type is harvested to produce rooibos tea. *A. linearis* can be found naturally in the dry and nutrient poor Cederberg and Suid Bokkeveld areas, South Africa (Dahlgren, 1968); it is cropped to produce the rooibos tea hence representing and important source of income for farmers in developing areas (Lötter et al., 2014).

Research interest in arid and semi-arid regions is increasing, especially in light of climate change and with the probability of aridification increasing in some regions (Classen et al., 2015). N and P are generally the limiting nutrients in nutrient-poor environments, and the acquisition and retention of these nutrients are thus important for plant growth and survival. Nitrogen can be made available to plants through symbiotic interactions or through the degradation of organic compounds by free-living soil microbes (Zogg et al., 2000). To retain nutrients, nitrogen can be resorbed from leaves before sequestration. Both plant and microbial physiological processes can be influenced by environmental parameters, and the acquisition and retention of nutrients may subsequently vary seasonally (Bever et al., 2010; He and Lamont, 2010). Strategies of nitrogen acquisition may also vary between wild populations and cultivated crops optimized for production aimed at commercial purposes. The variation in soil microbial community activity and microbial nutrient cycling in the Fynbos region, especially the nutrient-poor and dry Cederberg, has not been assessed.

As a legume in the Cederberg, *A. linearis* will also be influenced by changes in soil microbial nutrient cycling. Little is known about the effects of drought stress or the seasonal variability in productivity and nutrient cycling in this commercially valuable legume. In order to fully understand the influence environmental changes on the legume *A. linearis*, it is important to first assess the seasonal variations in soil microbial nutrient cycling (Koranda et al., 2013), and the consequent effects on N nutrition in cultivated and wild *A. linearis* legumes plants between seasons.

Owing to the seasonally dry climate, it is imperative to understand whether nutrient availability can become more limiting during drier seasons in the already-dry and nutrient-poor Cederberg. The aim of the present study was therefore to investigate the different functional strategies of N nutrition of cultivated and wild *A. linearis* plants, during the wet and dry seasons. The research focuses on the differences in plant-soil N cycling via plant N partitioning, soil microbial activity and BNF between cultivated and wild *A. linearis* plants.

We analysed the N nutrition strategies in wild and cultivated plants of *A. linearis* during the wet and dry seasons by comparing rates of BNF, leaf N and percentages of N re-absorption in plants and soil microbial activities.

2. Materials & methods

2.1. Study site

All samples were collected near Heuningvlei, Cederberg Mountains, South Africa, from wild and cultivated *A. linearis* populations (32.2°S, 19.13°E, 858 m asl). *A. linearis* (N.L.Burm.) R.Dahlgren plants ('shrub' form) growing among other fynbos plants was used to sample wild plants. Cultivated plants have undergone a regular harvesting cycle and are planted in rows, 2 m apart. Soil between cultivated plants is not covered by other vegetation (Fig. 1). The soils of the Cederberg area are highly leached acid sands, which are poor in nutrients and are characterized by a low water retaining capacity (Barnard and Greeff, 1993).

2.2. Sample collection

Ten mature shrubs of similar size were randomly selected in both sites, thus 20 shrubs in total were sampled. Sampling was completed in late summer (dry season) and in early winter (wet season) of 2014. The same shrubs were sampled during each season.

Twenty cm of undamaged and disease-free branches was cut off the top of each shrub and placed in paper bags. Leaf litter was collected at the base of each plant. Three soil samples (each 10 cm deep) were taken from underneath each shrub, equally spaced from each other. These three samples were pooled to form a combined sample for each shrub. Another three soil samples (each 10 cm deep) were taken 1 m away from each shrub and again combined to form a single sample for each plant. Soil samples were kept at 4 °C until analysed. For the leaf N re-absorption efficiency during each season, fresh leaves were collected from the green foliage and freshly senesced leaves from the surface below the canopy.

2.3. Plant and soil analysis

a) Stable N isotope analyses were conducted to determine symbiotic nitrogen fixation, via the ratio of $^{15}N/^{14}N$, henceforth referred to as $\delta^{15}N$. Percentage N was calculated for leaves and leaf litter for each respective sample. Percentage N re-absorption was taken as the difference between leaf N and leaf litter N for each pair of samples.

Plant samples were dried and then weighed into tin cups to an accuracy of 1 µg on a Sartorius micro balance (Thermo Scientific, Bremen, Germany). A Flash 2000 organic elemental analyzer was used to combust the samples and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (University of Cape Town, Archaeology Department). All three items are made by Thermo Scientific, Bremen, Germany. The in-house standards, i.e. Merck Gel, lentil and *Acacia saligna*, were used. All the in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen (Brenna et al., 1997).

b) The percentage N re-absorption efficiency was calculated as originally described by Killingbeck (1996), and recently used by Van der Colf et al., (2017) on legumes from the same ecosystem.

$$(N_{freshleaves} - N_{senescentleaves}/N_{freshleaves}) * 100$$

c) β-Glucosidase and urease that are secreted by soil microbes give an indication of the microbial activity in cycling C and N. Soil β-Glucosidase and urease activity was determined using the procedures of Eivazi and Tabatabai (1988) and Kandeler and Gerber (1988) respectively. Soil percentage ammonium N, percentage C and P content in mg/kg was determined according to the methods described by Barnard et al. (1990).

2.4. Data analysis

Prior to analyses, homogeneity of variance and the normality of data were tested before ANOVA to ensure the assumptions of ANOVA were met. Data were analysed using an analysis of variance (ANOVA) and where the ANOVA revealed significant differences between treatments, the means (n = 10) were separated using a post hoc Tukey's HSD, multiple-range test based on a significance level of 0.05. Kaleidagraph for Macintosh (Synergy Software, USA) and SuperAnova for Macintosh (Abacus Concepts, USA) were used for data analyses. Different letters indicate significant differences between treatments.

3. Results

For wild *A. linearis* plants, BNF was significantly higher ($p < 0.001$) than in cultivated plants, a pattern that is observed both in the dry and in the wet season (Fig. 2). BNF in the wild plants reached values of 80 and 91% for the dry and wet seasons, respectively. Overall BNF was greater in the wet season than in the dry one for the two plant varieties. The observed difference in BNF between cultivated and wild plants do