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Cultivation may not dramatically alter rhizobial community diversity or structure associated with rooibos tea (*Aspalathus linearis* Burm.f.) in South Africa

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A B S T R A C T

Rooibos tea, *Aspalathus linearis* (Burm.f.), represents one of South Africa's most important indigenous crops, and monotypic plantations are rapidly replacing wild populations. Dwindling wild rooibos populations may drastically reduce possible, but hitherto overlooked, natural resources to bolster commercial productivity and long-term sustainability.

Here, using next generation sequencing data, we seek to determine whether cultivation impacts on the diversity and community structure of mutualistic nitrogen fixing bacteria (rhizobia) associated with rooibos plants. We do this by generating operational taxonomic units (OTUs) from nodulation (*nodC*) and nitrogenase (*nifH*) DNA sequence data from rhizobia within root nodules of rooibos plants from cultivated and wild populations. For these rhizobial communities we found significant differences for various OTU diversity metrics due to geography (site), the interaction between site and status (cultivated vs. wild), but not for status. We also sequenced *nodC* soil microbiomes and found evidence for compositional differences in soil rhizobial communities due to geography, status (cultivated vs. wild), and the interaction between geography and status.

Our data adds to existing evidence suggesting that tea cultivation in South Africa may have limited impacts on soil microbial community diversity and structure, and that such changes are mainly driven by larger geographic processes rather than human-mediated disturbances such as cultivation.

1. Introduction

South Africa's Core Cape Subregion (CCR, previously Cape Floristic Region, and now forming part of the Greater Cape Floristic Region) is a global biodiversity hotspot, being home to more than 9000 species confined to an area of just over 90,000 km^2 (Manning and Goldblatt, 2012). Much of this diversity is endemic and phylogenetically unique (Cowling et al., 2009). The region's patchy habitats, as afforded by heterogeneous geology and varying soil nutrient conditions, are characterised by exceptionally high beta diversity turnover (Ellis et al., 2014). The CCR's hyper diverse flora is mirrored by a diverse ethnobotanical history that has, in some instances, developed into large-scale commercial endeavours (van Wyk, 2002).

Aspalathus linearis (Burm.f.), commonly known as rooibos tea, is an endemic CCR legume with a wide geographic range spanning parts of South Africa's Western Cape and Northern Cape Provinces (Dahlgren, 1968; Fig. 1). Utilisation of rooibos dates back centuries to when traditional Khoisan people harvested young shoots to make beverages (Morton, 1983). Rooibos has subsequently been developed into a major South African enterprise (Joubert and De Beer, 2011), mainly as caffeine-free teas popular for their unique phenolic constituents and flavours (Joubert et al., 2008). In response to a rising global demand for rooibos tea, commercial monotypic cultivation has increased while wild-harvested rooibos supplies dwindled (Malgas et al., 2010). Currently, only few small-scale farmers utilise wild rooibos populations as 'niche' products sold as unfermented, certified organic and fair trade (Joubert et al., 2008), and/or environmentally sustainable teas (Arendse and Oettlé, 2001).

Interactions between plants and soil microbes are important regulators of plant productivity and therefore have major consequences for the functioning of both agricultural and natural ecosystems. For example, like many legumes, rooibos successfully forms mutualistic symbioses with nitrogen fixing bacteria known as rhizobia. Rhizobia are free-living microbes that infect the root system (or more rarely stems) of host plants to form specialised structures known as root nodules. Within root nodules rhizobia fix atmospheric nitrogen, providing host plants with utilisable organic forms of the element while receiving nutrients from host plants in return (Franche et al., 2009). The relationship between rhizobia and legumes is generally not random. That is, successful nodulation is strongly influenced by the occurrence of 'compatible' co-evolved rhizobia in soils, and their absence or rarity

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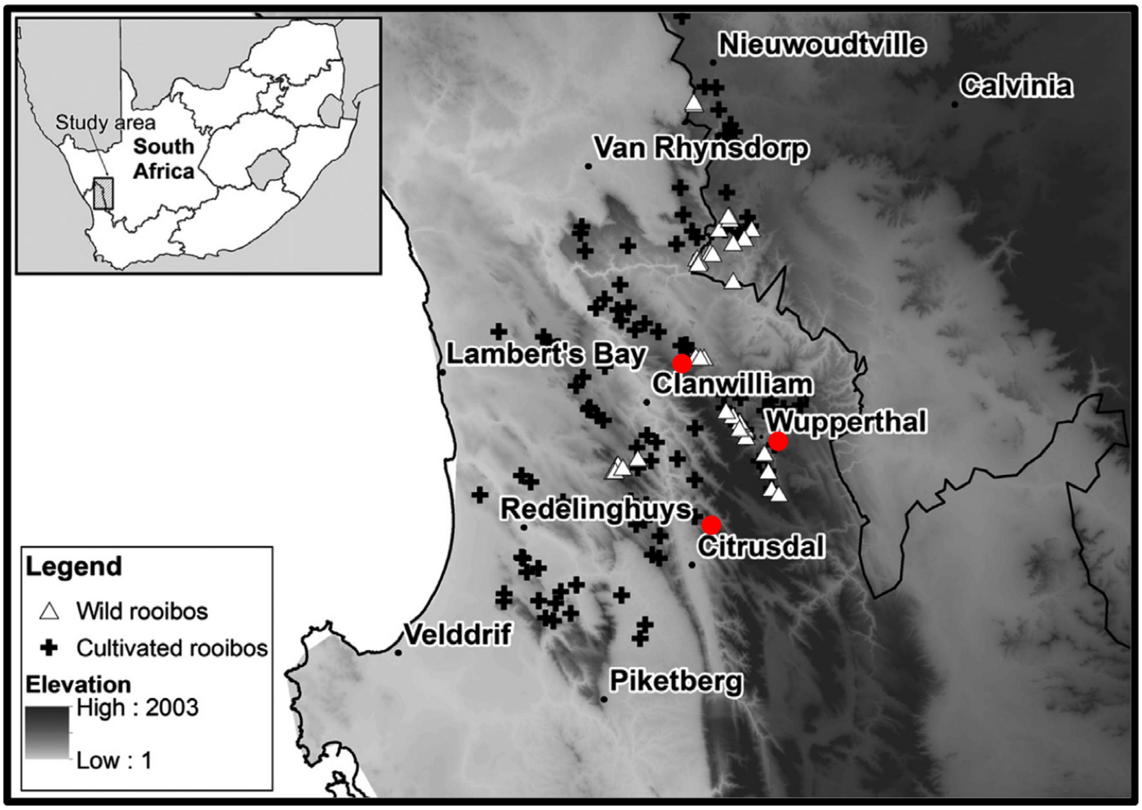

Fig. 1. Map illustrating previously surveyed locations of wild and cultivated rooibos populations (modified with permission from Lötter and Le Maitre, 2014) spanning the area from Nieuwoudtville (Northern Cape Province) to Piketberg (Western Cape Province). Paired sampling sites for the current study are indicated by red circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

may thus constrain the persistence of legumes, especially under novel environmental conditions (e.g. Béna et al., 2005).

There is increasing evidence that human activities cause major changes to the composition of soil microbial communities, and thus to the health, functioning, and productivity of soils. For example, monoculture farming is expected to lead to dramatically altered diversity and richness of soil microbial communities and therefore plant–microbial interactions (Lauber et al., 2008). This may be particularly true for rhizobia, as symbiotic efficiency and effectiveness is not only dependent on the availability of suitable host plants, but also abiotic conditions like soil nutrients (Pankhurst, 1981), pH (Ferguson et al., 2013) and temperature (Zahran, 1999). Tracking changes in soil plant–microbial interactions associated with crop domestication is therefore an important first step in sustainable crop development. At the same time, understanding the impacts of conventional farming approaches on soil microbial diversity can serve as a proxy for long-term agricultural sustainability.

Here, using next-generation sequencing data, and targeting nitrogen-fixing bacteria from root nodule communities and soils of cultivated and natural stands of rooibos tea, we tested the hypothesis that cultivation impacts on rhizobial communities. Specifically we tested two predictions from this hypothesis 1) that rhizobial diversity should be higher in soils, and therefore nodules, in wild rooibos populations than in monoculture plantations, and 2) that wild and monoculture populations should harbour compositionally different soil and nodule rhizobial communities.

2. Materials and methods

2.1. Study taxon

In the wild, *Aspalathus linearis* exhibits five distinct ecotypes all of which are relatively widespread throughout the CCR (Malgas et al., 2010). These ecotypes differ genetically and in their phenolic constituents (Van der Bank et al., 1995, 1999; Malgas et al., 2010) and exhibit unique life-history traits closely matched to fire survival strategies. The latter includes the 'erect' and 'tree' forms that regenerate through reseeding, while the 'prostrate' and 'shrub' forms resprout after fire (Malgas et al., 2010; Hawkins et al., 2011). A single high-yielding cultivar, the tree-ecotype 'Nortier', is widely planted and used for commercial production under monoculture conditions (Hawkins et al., 2011).

2.2. Sample collections

In order to characterise soil rhizobial communities of, and rhizobia associated with, wild and cultivated rooibos populations, we identified cultivated and wild sites in close proximity. Three such paired sites were identified near Citrusdal (Nooitgedacht farm; 32.28133S, 18.91442E), Clanwilliam (Klipopmekaar farm; 32.17786S, 18.806E) and Wupperthal (Wupperthal Rooibos Cooperative; 32.15463S, 19.10333E) in the Cederberg mountain region of South Africa (Figs. 1, 2). Three paired sites represent a low sampling effort, but finding wild rooibos stands in close proximity to cultivated fields proved problematic during this study. Soil samples were collected from underneath three randomly selected rooibos individuals at all sites (n = 18, three samples from cultivated and wild population pairs at three sites). Collected soils were kept in a cooler box with ice bricks during transportation, and once in the laboratory, stored at −80 °C until DNA extractions. At each site we also collected five root nodules from ten randomly selected individual rooibos plants (total nodules n = 300, 50 nodules from cultivated and wild population pairs at three sites). Root nodules were desiccated and preserved on silica until further use.

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