Rivalry for Nutrient Resources:

Is there competition belowground between leguminous trees and grasses, in a mesic and an arid savanna in the Kruger National Park?

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Abstract

As described in the resource-based co-existence theory, trees and grasses are able to co-occur due to partitioning of the edaphic environment in savannas. This study describes the fine root-distribution of dominant leguminous C_3 trees and C_4 grasses relative to soil nitrogen, phosphorus and water profiles using stable carbon and nitrogen isotope ratios (of the fine roots). The study occurs on a mesic savanna (737 mm MAP) site on sandy-loam soils and an arid savanna (547 mm MAP) site on clay-rich soils in the Kruger National Park, South Africa. We show that most tree and grass roots are located in the upper layers of the soil and both are present to the bottom of the profile. Root biomass is positively correlated to soil nitrogen and phosphorus and negatively to soil moisture and there were significant differences between sites, but very few of the results were significantly different down the soil profile. Therefore, the niche-separation hypothesis was not supported. The Scheiter & Higgins (2007) model illustrates that even though rooting niche separation is not an essential precondition for grass–tree coexistence, competition in the rooting zone can shape patterns of tree dominance in savannas, which may help in dealing with the problem of bush encroachment in savannas.

Introduction

Savannas are characterized by the co-dominance of trees and grasses (Knoop & B. H. Walker, 1985; Sankaran, Ratnam, & N. Hanan, 2004) with much speculation on how and why it's possible. This consistent co-existence of two very different components is seen under many environmental conditions with considerable structural variation from arid shrublands through lightly wooded grasslands to deciduous woodlands and dry forest (Walter, 1971; Knoop & Walker, 1985; Sankaran et al., 2004). For years, this has puzzled ecologists and has still not been fully answered, though many experiments and studies have been carried out and several theories put forward.

There are two main hypotheses concerning tree-grass co-existence in savannas; one resource-based and the other, disturbance-based. The former concerns partitioning of the rooting niches, where trees and grasses select their water and nutrients from different sections of the soil profile; grasses from the top layers, trees the deeper layers (the two-layer hypothesis) (Walter, 1971). It has long been thought that trees are able to coexist with grasses and shrubs through competition avoidance at the root level (Walter 1971; Walker & Noy-Meir 1982). The latter hypothesis concerns limitation of tree establishment by grazing, drought and fire (Bond, 2008; February & Higgins, 2010). Recently models have been trying to incorporate both and have shown that together one gets a better estimation of productivity and co-existence (e.g. Scheiter & Higgins, 2007). To quantitatively estimate plant competition is difficult due to the large number of morphologic-physiological characteristics of plants involved (Walter, 1971).

Root competition is a reduction in the availability of a soil resource to roots that is caused by other roots (Schenk, 2006). Trees compete with grass for both nutrients and water (Schenk, 2006; Scholes & Archer, 1997) as detailed for both adult trees (Belsky, 1994; Knoop & B. H. Walker, 1985) and seedlings (Cramer et al., 2007). Traditionally, water was thought the most influential aspect, with nutrients a lesser but still important factor (Walter, 1971; Walker & Noy-Meir, 1982; Belsky, 1994). Recently, research has turned to nutrients being the more influential in determining how trees and grasses interact (McCulley et al., 2004; February & Higgins, 2010; February et al.,2011).

One of the most limiting factors in a terrestrial ecosystem is nitrogen (available organically and inorganically) (Scholes & Walker, 1993). The largest nitrogen pool of a terrestrial ecosystem is the organic nitrogen (N) in the soil which is spatially variable, both horizontally and with depth (Scholes & Walker, 1993). The ratio of the stable isotopes ^{13}C and ^{12}C in carbon and ^{15}N and ^{14}N in nitrogen relative to a standard expressed as $\delta^{13}C$ and $\delta^{15}N$ respectively are still some of the most useful ways of measuring nutrients and the ecophysiological processes of plants (Dawson et al., 2002). For example, savanna trees have $\delta^{13}C$ values of -26.5‰ and grasses values of -12.5‰ (Dawson, 2002, February et al, 2011). Several studies have used these differences in isotope ratios to distinguish between tree and grass roots (Dawson, 2002; February & Higgins, 2010; February et al., 2011) due to the different photosynthetic pathways used; trees C_3, grasses C_4. A recent study has shown that in the savanna of the Kruger National Park the highest root biomass for both trees and grasses are in the top 20 cm of the soil (February & Higgins, 2010), and several studies have shown that the highest concentrations of nutrients are also in the top layers of the soil (Coetsee, February, & Bond, 2008; February & Higgins, 2010; February et al., 2011).

The ratio $\delta^{15}N$ can be used to understand the dominant biogeochemical processes happening in the soil (Scholes & Walker, 1993). However, only a few studies have shown how savanna trees and grasses relate to the $\delta^{15}N$ profile of the soil, as water has been assumed as the most influential factor. $\delta^{15}N$ values usually increase (enriched in the heavy isotope or more positive relative to zero) in depth in forest, savanna and grassland soils through mycorrhizal activity at the surface, loss to the atmosphere and other processes, with a slight decline at greater depths due to the influence of leaching (February & Higgins, 2010; Hobbie & Ouimette, 2009). But nutrients other than nitrogen (N) may be influencing root distributions in savanna (Craine, Morrow, & Stock, 2008). Therefore the ability of legumes, which are prominent in savannas, to fix N^2 may liberate them from N constraints, but other nutrients, such as phosphorus (P), may become a constraint (Cramer et al., 2007) though results are inconsistent in this regard. Total N does not, however, reflect the availability of inorganic N for plant uptake, which depends on the rate of N mineralization and the rate of loss from the soil as a consequence of consumption by the biota and leaching/volatilization (Cramer et al., 2007). Generally there is an inverse relationship between $\delta^{15}N$ and nutrient availability, i.e. nutrient rich ecosystems tend to be isotopically enriched (Vitousek & Walker, 1989), as plants can afford to discriminate when the concentration of N in the soil is high relative to the plants N requirements. So soil and plant $\delta^{15}N$ enrichment has been associated with areas that experience lower precipitation (McCulley et al., 2004).

Research on the partitioning of the soil resources (focussing mainly on water) has not reflected the two-layer hypothesis to any great degree, as root mass is a poor indicator of actual root activity (Kulmatiski et al., 2010). So competition between the roots of trees and grasses is still thought to be part of the explanation of co-existence, especially during the seedling stage of the trees (Cramer et al., 2007). Exactly where trees and grasses take up their nutrients from has not yet been resolved, as direct root activity measurements are rare.

In this study I will focus on root activity of mature trees (C_3) and grasses (C_4) at a detailed scale down the soil profiles of a mesic and arid savanna in the Kruger National Park. Plants adjust their fine root mass distribution to access uptake of a limited resource whether nutrients or water (Schenk & Jackson 2002). If surface layer water is limited, then high concentration of roots should be found in the deep layers where water is more available (Schenk & Jackson, 2002; February et al., 2011). Alternatively, if nitrogen and phosphorus are limiting we would expect roots closer to the soil surface, since nutrients are concentrated in the surface soil horizons (February & Higgins, 2010). The mesic site should have lower nutrient availability and therefore higher $\delta^{15}N$ values than the arid site (Moore, 2007, *unpublished*), but the same pattern should occur in both sites.

Investigation of changes in soil nitrogen and phosphorus with depth and comparing to the fine root biomass of trees and grasses should show whether there is niche separation between them and if it is determined by concentrations of nitrogen and phosphorus, rather than water (February et al., 2011). This should add to our understanding of edaphic processes that may influence tree- grass coexistence in mesic and arid savanna systems.

Methods

Study sites

The two sites that we consider, Satara (31.77'E, 24.40'S) and Pretoriuskop (31.14'E, 25.08'S), are located respectively in the central and southern section of the Kruger National Park in South Africa. Hot wet summers and dry mild winters typify the region's climate with mean annual precipitation 737 mm at Pretoriuskop and 547 mm at Satara. Mean monthly maximum and minimum temperatures are 26.3°C and 17.5°C at Pretoriuskop and 29.8°C and 16°C at Satara. Rain falls in the summer months caused by convection storms or tropical cyclones. The distinct seasonality of the rainfall results in a growing season that starts with the first rains in late October and continues to the end of the rains in April (February et al., 2011;Venter et.al., 2003).

I sampled the most common tree and grass from two study sites, one at Pretoriuskop and the other at Satara. At Satara, a fine-leaved open savanna [Granite Lowveld Savanna (Mucina & Rutherford, 2006)], the dominant tree species is the legume *Acacia nigrescens* and the dominant grass *Panicum maximum.* The soil at Satara is nutrient-rich clay of the Letaba formation basalts formed from the Karoo supergroup (Venter et al., 2003; Mucina & Rutherford, 2006). The sandy nutrient-poor soils at Pretoriuskop (Barton et al., 1986) are derived from the underlying Nelspruit granite (migmatite, gneiss and granite) and support a broad leafy savanna (Pretoriuskop Sour Bushveld (Mucina & Rutherford, 2006)) with dominant tree species *Terminalia sericea* and grass species *Hyperrhenia filipendula* (February & Higgins, 2010).

Field sampling and laboratory analyses

Soil pits were dug directly under the canopy (south/shady side) of five mature trees (3+ m in height) approximately 50cm from the trunk of the most common species at each site (*Acacia nigrescens* in Satara and *Terminalia sericea* in Pretoriuskop) and between tussocks of a common grass (*Panicum maximum* in Satara and *Hyperrhenia filipendula* in Pretoriuskop). Sampling was carried out at the onset of the dry season in June 2011. Five replicate soil pits were dug as far as down as possible at each of the two sites. 20 x 20 cm layers of soil were taken at depth increments of 1, 5, 10, 15, 25 cm and a further 10cm if possible (total depth was 19 cm at Satara and 32 cm at Pretoriuskop). Each layer was individually placed in separate plastic bags and sealed with adhesive tape. At each pit, the roots of each dominant tree (C_3) and grass (C_4) were also sampled. These samples were used as end member values representing the trees and grasses at each site.

A 10g subsample of each soil sample was removed, weighed (to the nearest 0.1g using a ScoutPro OHAUS) and oven-dried at 60°C for 24 hours before reweighing to determine soil moisture content. Carbon content was determined after incineration of the subsample at 500°C (kiln) and then reweighed. From the rest of the soil, all the fine root matter (>2mm in diameter) was separated by dry-sieving through a 500 μm sieve. Fine roots are the effective absorbing root surface and are primarily responsible for ion uptake (De Koon & Visser, 2003). Total weight of the soil after sieving was recorded and 100g subsample removed from each sieved sample. Available phosphorus was analysed testing 3.3g of soil with 25ml Bray II, reacted with Malachite Green and tested with colour spectrophotometry, and percentage total nitrogen through mass spectroscopy.

All the root matter was washed, oven-dried to constant weight at 55°C and ground to a fine powder using a Retsch MM200 ball mill (Retsch Inc. GmbH&Co KG, Haan, Germany), isotopically determining $\delta^{15}N/^{14}N$ and $\delta^{13}C/^{12}C$ ratios through use of a Thermo Finnigan Delta plus XP Mass Spectrometer coupled with a conflo III device to a Thermo Finnigan Flash EA1112 Elemental Analyser with automatic sampler (Thermo Electron Corporation, Milan, Italy), and for total phosphorus content using the dry-ash method (samples were ashed, taken up in acid and analysed by use of an inductively coupled plasma - optical emission spectrometer (ICP-OES) against suitable standards).

Stable isotope values are given as;

$\delta(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$

where R is the standard ratio of the heavy to light isotope ($\delta^{15}N/^{14}N$ and $\delta^{13}C/^{12}C$). The values are expressed relative to a standard, atmospheric air for Nitrogen and Vienna Pee Dee Belemite for Carbon. Higher δ values indicate enrichment relative to the heavier isotope (either ^{15}N or ^{13}C). The deviation from the standard is denoted by δ and the results expressed in parts per thousand (‰) (Dawson et al., 2002).

I used the $\delta^{13}C$ values of the total root sample and the mean $\delta^{13}C$ values of the end member fine roots (tree, C_3 and grass, C_4) to determine the relative proportion of C_3 and C_4 derived carbon in a root sample. C_4 plants have $\delta^{13}C$ values of approximately -13.6% while C_3 plants have $\delta^{13}C$ values of -27.4%. These values are comparable to previous studies (e.g. February & Higgins, 2010). To obtain an estimate of how the proportion of tree and grass root material changes with depth, I assume that the isotopic signal of the root sample (S) is a simple mixture of the isotopic signatures of the grass (G) and tree (W) material in the sample. A mixing equation/model represents this assumption;

$S = pW + (1\text{-}p)G$

where p is the proportion of the sample that is tree root and $1\text{-}p$ is the proportion of the sample that is grass root. The end members provide estimates of W and G while the measured isotopic signature of the sample is S, hence the only unknown is p [$p = (G\text{-}S)/(G\text{-}W)$] (Dawson et al, 2002, February & Higgins, 2010, February et al., 2011).

Data analyses

When analysing the data from the soil profiles I acknowledge that samples taken from a single profile are not independent and that profiles are grouped (blocked) in sites. Therefore, in the statistical analysis I treat the factors profile and site as random effects (with profile nested within site) and soil depth as the fixed effect (February & Higgins, 2010).

A Student's t-test was performed to test significant differences between root $\delta^{13}C$ values as well as root $\delta^{15}N$ values for each of the two species at each site. Correlations were carried out between total root biomass and total soil N, available soil P and soil moisture. For further analyses, data were transformed using (log+1) to achieve normality. One-way analysis of variance (ANOVAs) were used to test for differences between root biomass, gravimetric water content, soil available P and soil total N along the soil profile. An ANOVA was also used to test whether the N(%) values for fine roots from the top two layers of the profile differed from values obtained for fine roots from the bottom layers and how these compared to the grass and shrub end member values. The same procedure was done for P (%) of the roots. Differences in root biomass with depth between species were tested using two-way analysis of variance. Tukey's HSD procedure was used to make paired comparisons for the variables at each sampled depth. Results are reported to mean values ± 1 standard error. All statistical analyses were performed using Statistica, V10 (Statsoft, Inc., 1984-2011) (February et al., 2011). In the analysis I tested for interactions between the main effects and between sites.

Results

The $\delta^{13}C$ values of the fine roots of the four species used in this study (*Terminalia sericea, Hyperrhenia filipendula, Acacia nigrescens* and *Panicum maximum,*) show that the grasses and trees use different photosynthetic pathways (C_3 for trees and C_4 for grasses). These results were significantly different from each other, allowing me to use mass balance equations to determine percentage of tree and grass roots through the profile. Fine root end member $\delta^{13}C$ values for *T.sericea* (Table.1) were significantly more depleted than *H. filipendula* (t(8)=35.47, p<0.001), and *A.nigrescens* were significantly more depleted compared to *P. maximum* (t(8)=40.67, p<0.001). Fine root end member $\delta^{15}N$ values for *T.sericea* (Table.1) were significantly more enriched than *H.filipendula* (t(8)=-4.56, p<0.001) and *A.nigrescens* significantly more enriched than *P.maximum* (t(8)=3.13, p<0.001). The tree and grass species at each site were significantly different from each other in their end member $\delta^{13}C$ values and $\delta^{15}N$ values (Table.2), except for the grasses' end member $\delta^{13}C$ values.

Root Biomass and Soil Factors

The results from the mass balance equation show that at both sites, both tree and grass roots were present throughout the soil profile (Fig 1). At the granite site (Pretoriuskop), there were significantly higher amounts of root biomass in the top two layers (mean = 2060 g/m^3) compared to the bottom layers (mean = 480 g/m^3) ($F_{5, 22}$ = 38.66, p<0.001). The same occurred in the clay site (Satara) but only when comparing the surface layers (mean = 2700g/m^3) to the bottom layers (mean = 790g/m^3) ($F_{4, 18}$ = 4.57, p=0.01). There were no significant differences in root biomass between sites or between tree and grass in either site. Root biomass decreases rapidly between 1- 15cm down the profile at the Pretoriuskop site and between 1-5cm at Satara, staying at ~250 g/m^3 and ~500 g/m^3 respectively (Fig.1).

Correlation analysis revealed significant (p<0.05), strong positive correlations between total root biomass and total soil nitrogen (r= 0.76 for Pretoriuskop and r=0.55 for Satara) and for available soil phosphorus for Pretoriuskop (r= 0.83) but an insignificant weak correlation for Satara (r= 0.38). Total root biomass was significantly negatively correlated to soil moisture for Pretoriuskop (r= -0.74) but insignificantly for Satara (r=0.40). Overall there are much stronger relations between root biomass and the three soil factors for the mesic granite-based savanna (Pretoriuskop) than the arid clay-based savanna (Satara).

Soil moisture increased down the profile and was significantly lower in the top two layers of the soil compared to the bottom layers for the granite soil at Pretoriuskop ($F_{5, 22}$ = 14.55, p <0.001) and for the clay soil at Satara ($F_{4, 18}$ = 7.56, p<0.001) (Fig.2a). The granite soils at Pretoriuskop (2-4%) had significantly lower soil moisture content ($F_{9, 36}$ = 3.35, p<0.001) than the clay soil at Satara (7-11%) (Fig.2a).The surface layer moisture level (0-1cm) was significantly different from the bottom layer (25-32cm) both at Pretoriuskop ($F_{5, 22}$ = 14.55, p <0.001) and Satara ($F_{4, 18}$ = 7.56, p<0.001).

Carbon content decreases gradually down the soil profile for Satara from 8.5-6.5% (Fig 2b), whereas Pretoriuskop increases from 2.1-3.0% down to 8 cm then decreased to 1.89 % at 10cm, decreasing only slightly further for the rest of the way down. Carbon content is significantly different between sites for all the layers except the surface layer ($F_{9, 36}$ = 3.35, p<0.001). There is no significant difference between any of the layers for Pretoriuskop and only between the surface layer and the deeper layers for Satara ($F_{4, 18}$ = 5.54, p <0.004).

Nitrogen content decreases down the soil profile for both sites (Fig.3). Pretoriuskop soil nitrogen values were much lower than Satara and ranged from 0.085% at the surface, to 0.06% at 5cm then decreased to 0.04% from 15-32cm (Fig.3a). There were significant differences between the surface layer and the bottom layers ($F_{5, 22} = 19.26$, $p < 0.001$). At Satara only the surface layer was significantly different from the layers below ($F_{4, 18} = 8.88$, $p<0.001$). Total soil nitrogen for Satara decreased from 0.23% in the surface layer to 0.18% at 5cm down, and was 0.15% at the deepest layer (Fig.3b) but none were significant. There were significant differences between Pretoriuskop and Satara between all layers except the surface ones ($F_{9, 36} = 3.35$, $p<0.001$).

There were significant differences in soil phosphorus between the two sites for all layers ($F_{9, 36} = 3.35$, $p<0.001$), as Satara values were much higher in available soil phosphorus (3.51 mg/kg compared to 22 mg/kg) (Fig.4). But there were no significant differences between layers in the granite soils of Pretoriuskop or the clay soils of Satara. Pretoriuskop showed a rapid decrease in the top 10 cm of the soil profile (3.51 – 2.58mg/kg) and changed little going deeper (Fig.4a). Satara decreased as well in the top 10cm, but gradually (from 22 -17 mg/kg), with a bigger decrease between 10cm and 15cm (17-12 mg/kg) (Fig.4b), and little change further down the profile.

Root characteristics

As the end member values were very different from those for the bulk root values (Table.1), contradictory to previous research (Cramer et al., 2007), the $\delta^{15}N$ aspect for root matter was discarded and root %N (Fig.5) was used instead to compare to soil N (Fig.3).There was significant difference for fine root %N values between the surface and bottom layers for Pretoriuskop ($F_{1, 26} = 11.63$, $p<0.002$), none between layers at Satara, but between sites there was significant difference at 15cm depth (Fig. 5; $F_{4,38} = 3.00$,$p<0.03$) which may be due to large standard error at that point. Fine root %N values decreased down the soil profile for both sites, 0.8 - 0.45% for Pretoriuskop (Fig.5a) and 1.21 -0.81% for Satara (Fig.5b). Satara showed a small increase at 15cm while Pretoriuskop decreased gradually all the way down the profile (Fig.5).

There were significant differences between top and bottom layers for % P found within the roots, both at Pretoriuskop ($F_{1, 26} = 9.94$, $p<0.004$) (Fig.6a) and at Satara ($F_{1, 21} = 7.46$,

$p<0.01$) (Fig.6b). There was only a significant difference between sites at 5-15cm layers down ($F_{9,36} = 3.35$, $p<0.02$). Root % P decreased at Pretoriuskop from 0.051% to 0.03% (Fig.6a) and increased from 0.09% to 0.092% at 5cm down then decreasing thereafter to 0.045% at Satara (Fig.6b).

Discussion

Between Sites

Trees and grass species of two different savanna systems were tested against the niche-partitioning theory hypothesized by Walter (1971). Are trees and grasses avoiding competition with each other by separating access to soil characteristics? The mesic savanna (Pretoriuskop) showed strong correlations between the root biomass and soil N, P and moisture (e.g. r= 0.76 for soil N), while the arid savanna (Satara) had similar but much weaker correlations (e.g. r= 0.55 for soil N). Biomass was highest where the nutrient concentrations for N and P were highest and decreased down the soil profile (compare Figs.3&4 with Fig.1).This decrease in soil nitrogen and soil phosphorus with depth is associated with the relatively high organic biomass close to the surface (2060 - 2700g/m^3) and a decrease in biomass with increasing soil depth (200 - 500 g/m^3) (February et al., 2011). This suggests that in the arid, nutrient-poor environment root distributions may be primarily responding to N and P availability rather than water availability as proposed by Walter (1971).

A previous fertilization study carried out in parts of the Kruger National Park on grasslands, showed that unfertilized vegetation across all their sites had N:P ratios which indicated that aboveground production was N-limited, but they showed their sites were actually consistently co-limited by N and P (Craine et al., 2008). Soil moisture and N and P were significantly different between Satara and Pretoriuskop in all layers except the surface ones (Fig.3 & 4). The basalt clay at Satara has much finer particles and holds higher amounts of water than the sandy granite soils of Pretoriuskop (Fig 2a). Granite soils have a smaller pool of nitrogen with high turnover rate whereas basaltic soils have a much greater pool with an overall slower turnover rate (Venter et al., 2003). The higher rainfall site had lower nutrient availability (compare Fig.3a & b and 4a & b) due to higher plant productivity and possibly increased loss through leaching (Belsky, 1994; Scholes & Walker, 2003; Cramer et al., 2007;

February & Higgins, 2010), therefore Pretoriuskop was expected to have higher root $\delta^{15}N$ values than the arid site, Satara (Moore, 2007). Generally there is an inverse relationship between $\delta^{15}N$ and nutrient availability, i.e. nutrient rich ecosystems tend to be isotopically enriched, as plants can afford to discriminate when the concentration of N in the soil is high relative to the plants N requirements (Vitousek & Walker, 1989). So soil and plant $\delta^{15}N$ enrichment has been associated with areas that experience lower precipitation (McCulley et al., 2004). This was supported by this study, where Satara (the arid site) had higher soil and plant %N (Figs.3 & 6).

Distinct decreases in concentrations of P (Fig.4) and other less mobile exchangeable cations (e.g. potassium, calcium and magnesium) from the top to bottom layers in sandy soils such as those at Pretoriuskop (Fig.4a), is probably because of larger amounts of organic material contained in the top layers (Fig.2b) (Venter et al.2003). The fine-root matter increases cation exchange capacity and therefore their capacity to retain soluble minerals (Venter et al., 2003). This implies that the fine roots of trees and grasses are partitioning according to access of nutrients rather than water in savannas, supporting earlier studies (February et al., 2011). As the highest percentage of root biomass is in the upper soil layer, the increase in soil moisture below this depth could be seen as a representation of an active process where evaporation and root uptake remove soil moisture from the upper soil layers (February et al., 2011). But root mass is a poor indicator of root activity (Kulmatiski et al., 2010),

Competition and disturbance based models assume that larger (post-sapling) trees are superior competitors to grasses, and that grasses have a minimal effect on the growth and survival of these trees (Scholes & Archer, 1997). Grasses are thought to be the controlling element in savannas in studies which have focussed on water supply (e.g. Walter, 1971; Knoop & Walker, 1985; Le Roux et al., 1995; Schenk, 2006; February & Higgins, 2010). Trees are predicted to be more dominant in loose, sandy soils and grasses to have the advantage in finer, clay soils with higher water holding capacity (Walter, 1971) as physical /chemical conditions of soils may inhibit the penetration of plant roots and volume of soils they use (Venter et al., 2003). But most of these studies were not related to the fine root matter (<2mm in diameter) where the effective absorbing occurs and are primarily responsible for ion uptake (De Koon & Visser, 2003). That in both sites, there was no displacement in the middle of the profile implies no escape from competition in these soil horizons (Belsky 1994; Scholes and Walker 1993, February et al., 2011). This adds to the suggestions that niche partitioning may not be sufficient to explain tree–grass coexistence in savannas (Sankaran, Ratnam, & Hanan, 2004; Riginos, 2009)

Tests for available phosphorus are notoriously inconsistent (Scholes & Walker, 1993). The available phosphorus pool is much more variable than the soil nitrate pool (Scholes & Walker, 1993). As this is a preliminary study in detailed terms of available soil phosphorus content for soil profiles, the sample results for phosphorus may not reflect the true amounts of available soil phosphorus. The large difference in available soil P between Pretoriuskop and Satara (3.51mg/kg compared to 22mg/kg) are similar to those found by Craine et al. (2007) (3.23mg/kg and 51.27mg/kg respectively). As the end member values for %P in fine root matter were not very different for *T.sericea* and *H.filipendula* at Pretoriuskop and *A.nigrescens* and *P.maximum* at Satara, this could explain why there were no significant differences between layers.

Within sites; Down the soil profile

To quantitatively estimate plant competition is difficult due to the large number of morphologic-physiological characteristics of plants involved (Walter, 1971). Roots compete for soil resources such as nutrients, water and space, and their availabilities may be reduced due to depletion or due to a variety of direct root interactions (Schenk, 2006).

Plants adjust their fine root mass distribution to access uptake of a limited resource (Schenk & Jackson, 2002). This was reflected in (Fig .1) where the highest amount of root biomass (2060 -2700 g/m^3) was found in the top 5cm of the soil at both the mesic (Pretoriuskop) and arid (Satara) savanna site, refining data from earlier studies (e.g. Le Roux, Bariac, & Mariotti, 1995; February & Higgins, 2010) and agreeing with findings from another recent study (February et al., 2011). However, contrary to February et al.'s (2011) findings which showed a separation of 80% shrub fine roots in the top 5cm of the soil and very little grass, this study showed tree and grass species to occur in almost equal parts down the soil profile at both a mesic and an arid site (Fig.1). This does not support the soil partitioning hypothesis where grasses possibly force trees to access their nutrients and/or water from the deeper layers (Walter, 1971, Walker & Noy-Meir, 1982) due the nature of their root systems. Resource uptake is affected more by the amount and spatial distribution of resource-acquiring organs, relative to the spatial distribution of resources (Schenk, 2006). Trees usually extend their roots laterally and horizontally very far compared to compact-rooted grasses and both are influenced by the texture of the soil (Walter, 1971).

The fine –root %N and %P results combined with the results for my mixing model indicates that *T.sericea* and *H.filipendula* at Pretoriuskop and *A.nigrescens* and *P.maximum* at Satara are not separating in rooting depth or resource acquisition within the same vegetation type. There were more significant differences between vegetation types than expected; probably due to the physical characteristics of the soils. e.g. the slight spike in soil %P at 15cm for Satara is reflected in % root P but only there, which could explain why there is little significant difference compared to Pretoriuskop (Fig.5). But within sites – neither supports the niche-separation hypothesis.

N and P ratios in vegetation have been offered as a simpler index of the limitation of N and P. Vegetation on granite soils, which has a higher N:P, should respond more to P addition or be more likely to respond to P alone than that on basalt soils (Craine et al., 2008). This would extend this study to include a temporal aspect but this would involve fertilization studies which are difficult to do on mature trees due to long time factor and large extent of the root systems. Widespread co-limitation between N and P, is more common than is shown by the simple N:P ratios in grasslands of Kruger (Craine, et al., 2008) and may be because trees and grasses can coexist only when intraspecific competition for soil resources is stronger than interspecific competition for soil resources (Scheiter & Higgins, 2007). Within current rooting niche models, grazing, browsing, and fire are not responsible for coexistence but rather serve to modify the relative abundance of grasses and trees (Scheiter & Higgins, 2007).

The decreased %N for trees grown with grass indicated that N^2 fixation was strongly enhanced by competition with grass for N in Cramer et al.'s green-house experiment (2007). N^2 fixation by legumes is often thought to be especially sensitive to P limitation as earlier studies showed that alfalfa N^2 fixation capacity increased with P addition rates (Cramer et al., 2007). However, that N^2 fixation is especially sensitive to limited P is not found consistently (Cramer et al., 2007). There is much evidence that grasses limit tree seedling establishment through competition for light, water and nutrients and by exposing tree seedlings to the hazards of fire (Scholes & Archer 1997). Grasses create greater levels of temporal variety of soil resources than the woody component in savannas (Mclaren, Wilson, & Peltzer, 2004).

The results from this study demonstrate that *T.sericea* and *H.filipendula* at Pretoriuskop and *A.nigrescens* and *P.maximum* at Satara have their fine-root matter growing where nutrient resources are highest. This is probably related to nutrients being deposited from aboveground biomass (litterfall, excrement of animals) and brought up from below through hydraulic redistribution, to the upper layers (February & Higgins, 2010; McCulley et al., 2004), allowing both the tree and grass species to access the limiting nutrients. However, the rooting niche assumption is not empirically supported here and in many savannas, yet grasses and trees coexist in these systems (Sankaran et al., 2005; Scheiter & Higgins, 2007). This observation suggests that the rooting niche hypothesis cannot be a general explanation for grass–tree coexistence. As reviewed by Sankaran et al. (2004), a number of alternative mechanisms have been explored. Notable here are models that propose that temporal and/or spatial variation in environmental conditions prevents grasses from excluding trees or trees from excluding grasses (Scheiter & Higgins, 2007).

The Scheiter & Higgins (2007) model illustrates that even though rooting niche separation is not an essential precondition for grass–tree coexistence, competition in the rooting zone can shape patterns of tree dominance in savannas (February & Higgins, 2010) Leguminous tree seedlings (C_3) utilize N^2 fixation to cope with intense competition from nitrogen-use efficient C_4 grasses for N while niche segregation (Walter 1971) for nutrient acquisition is impossible for that stage of the lifecycle (Cramer et al., 2007).

Understanding the tree-grass interactions within savannas has conservational importance in many parks today where woody/bush encroachment and its consequences for the biodiversity and economic productivity of savanna ecosystems, is becoming a problem (Riginos, 2009; Bond, 2008, Sankaran et al., 2005). Experimental studies have tested the effects of grasses on trees or incorporated these effects into models of tree demography (Sankaran, Ratnam, & N. Hanan, 2004) and these should help to understand what is going on. Future experimental research should focus on testing the effects of grasses on a variety of savanna tree species and demographic stages (including seedling survival and adult reproduction), for a variety of grass densities, and over a variety of edaphic and climatic conditions (Riginos, 2009). Directly comparing empirically derived estimates of the magnitude of the various determinants of tree demography using seedling survival and adult reproduction, will we further our understanding of tree–grass coexistence and the dynamics and management of savanna systems (Riginos, 2009).

Conclusions

These results advocate the usefulness of isotope measurements and simple mass equations in understanding edaphic processes between trees (C_3) and grasses (C_4),as root mass is a poor indicator of root activity (Kulmatiski et al., 2010). However, the modified two-layer hypothesis of Walter (1971) regarding tree-grass interactions, using nutrients instead of water was not supported. Mature trees in savannas provide a different picture compared to seedlings/saplings i.e. no clear indication of competition for nutrients. The Scheiter & Higgins (2007) model illustrates that even though rooting niche separation is not an essential precondition for grass–tree coexistence, competition in the rooting zone can shape patterns of tree dominance in savannas (February & Higgins, 2010). Bush encroachment is of great concern to many stakeholders these days (Bond, 2008), and understanding more how these two plant forms interact belowground would be very useful.

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Tables and Figures

Table 1: Isotopic values for the dominant tree and grass species (end member values) (N=5 for all values).

Species	**$\delta^{13}C$ values**	**$\delta^{15}N$ values**
Terminalia sericea	-27.71±0.19‰	1.46±0.37‰
Hyperrhenia filipendula	-13.34±0.41‰	-0.57±0.2‰,
Acacia nigrescens	-27.07±0.27‰	-0.72±0.65‰
Panicum maximum	-13.75±0.18‰	1.63±0.37‰

Table 2: Results from Student's t-test between sites for tree and grass species end member values (df = 8 for all values). Significant differences are indicated by *.

Species	**$\delta^{13}C$ values**	**$\delta^{15}N$ values**
Terminalia sericea/ Acacia nigrescens (Trees)	T= -2.00, p = 0.04 *	T = 2.76, p = 0.012 *
Hyperrhenia filipendula/ Panicum maximum (Grasses)	T = 1.00, p = 0.174	T = -5.25, p = 0.00039*

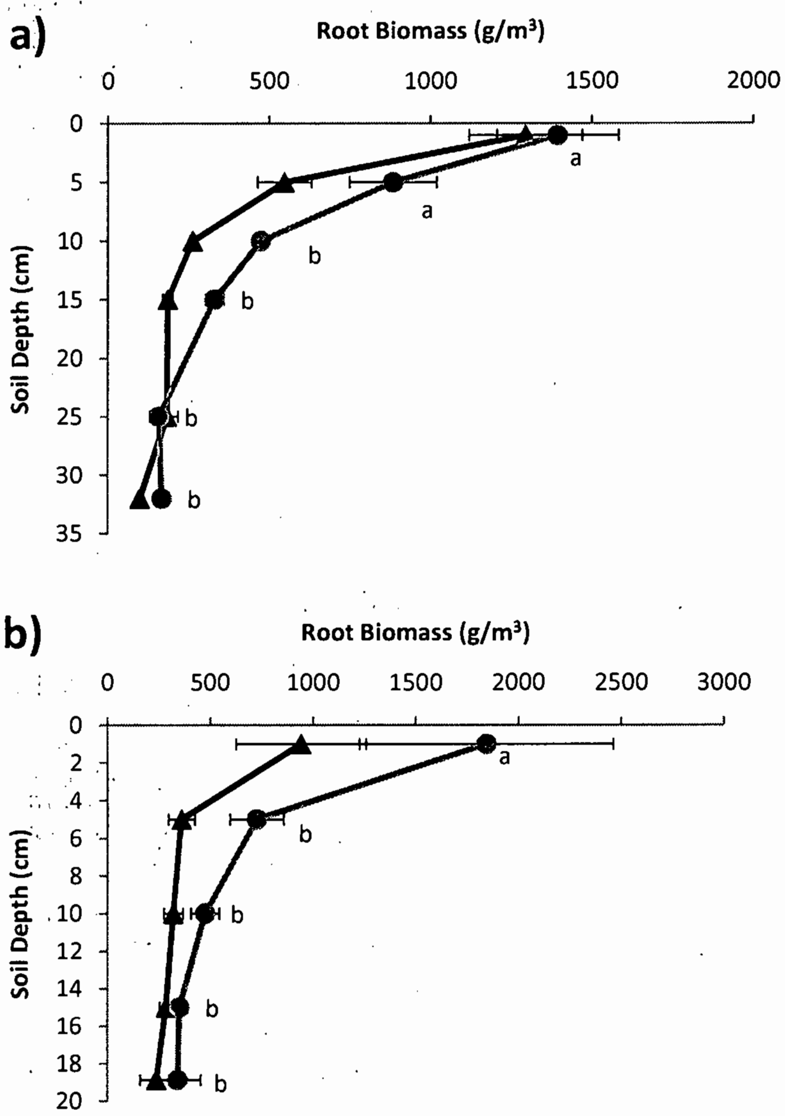

Fig 1: The vertical distribution of root biomass, mean ±SE, of both tree (▲) and grass (●) at (a) Pretoriuskop and (b) Satara. The different letters indicate the significant differences ($p<0.05$) for total root biomass down the soil profile. There were no significant differences between tree and grass at either site or between sites.

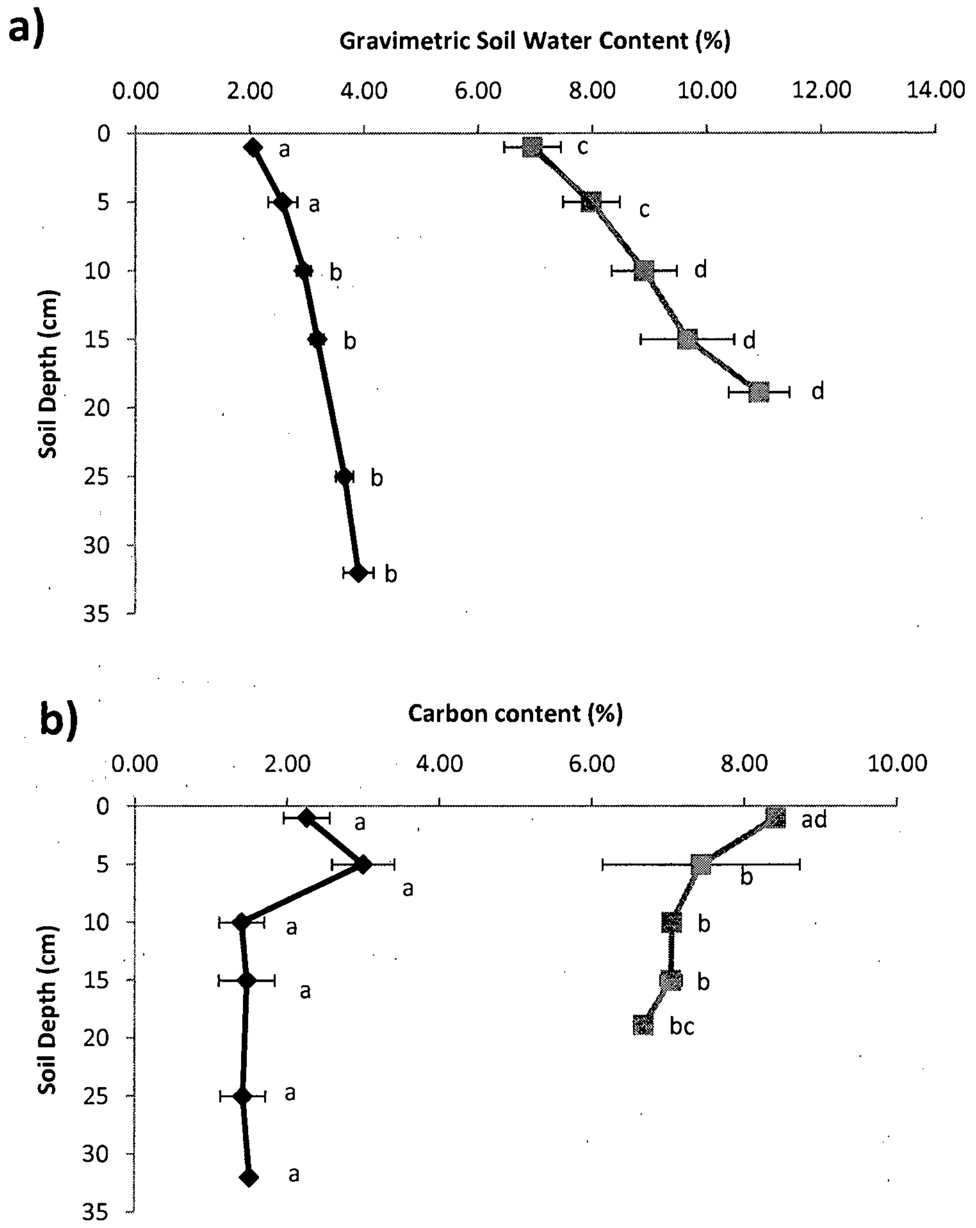

Fig 2: Vertical distribution of (a) soil moisture content (July 2011) and b) carbon content from Pretoriuskop (♦) and Satara (■).Values are means ± SE. Values with different letters indicate the significant differences ($p<0.001$) between layers and sites.

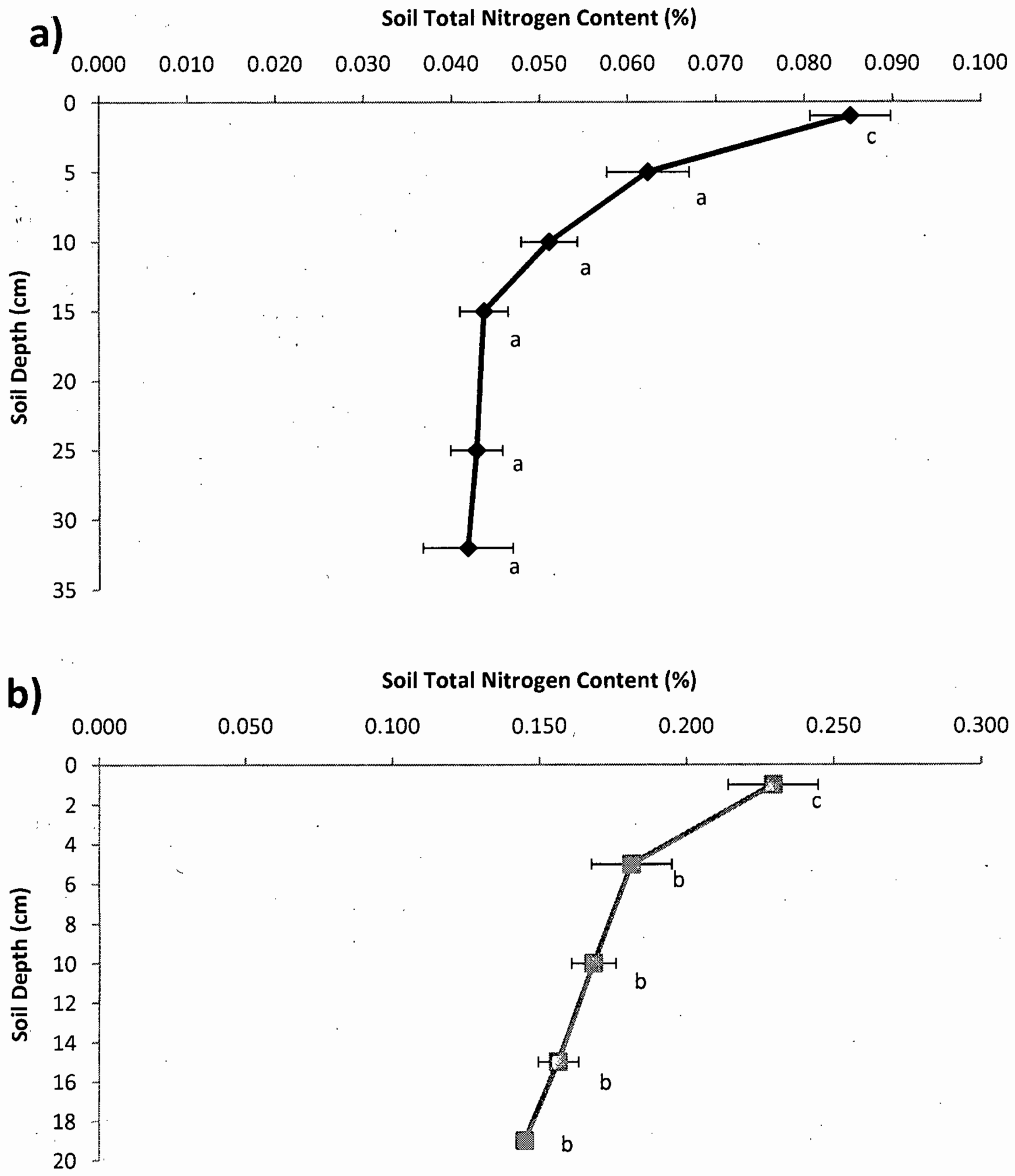

Fig 3: Total soil nitrogen content (±SE) down the soil profile a) Pretoriuskop (♦) and b) Satara (▪).Values are means ± SE. Values with different letters indicate the significant differences (p<0.001) between layers and sites.

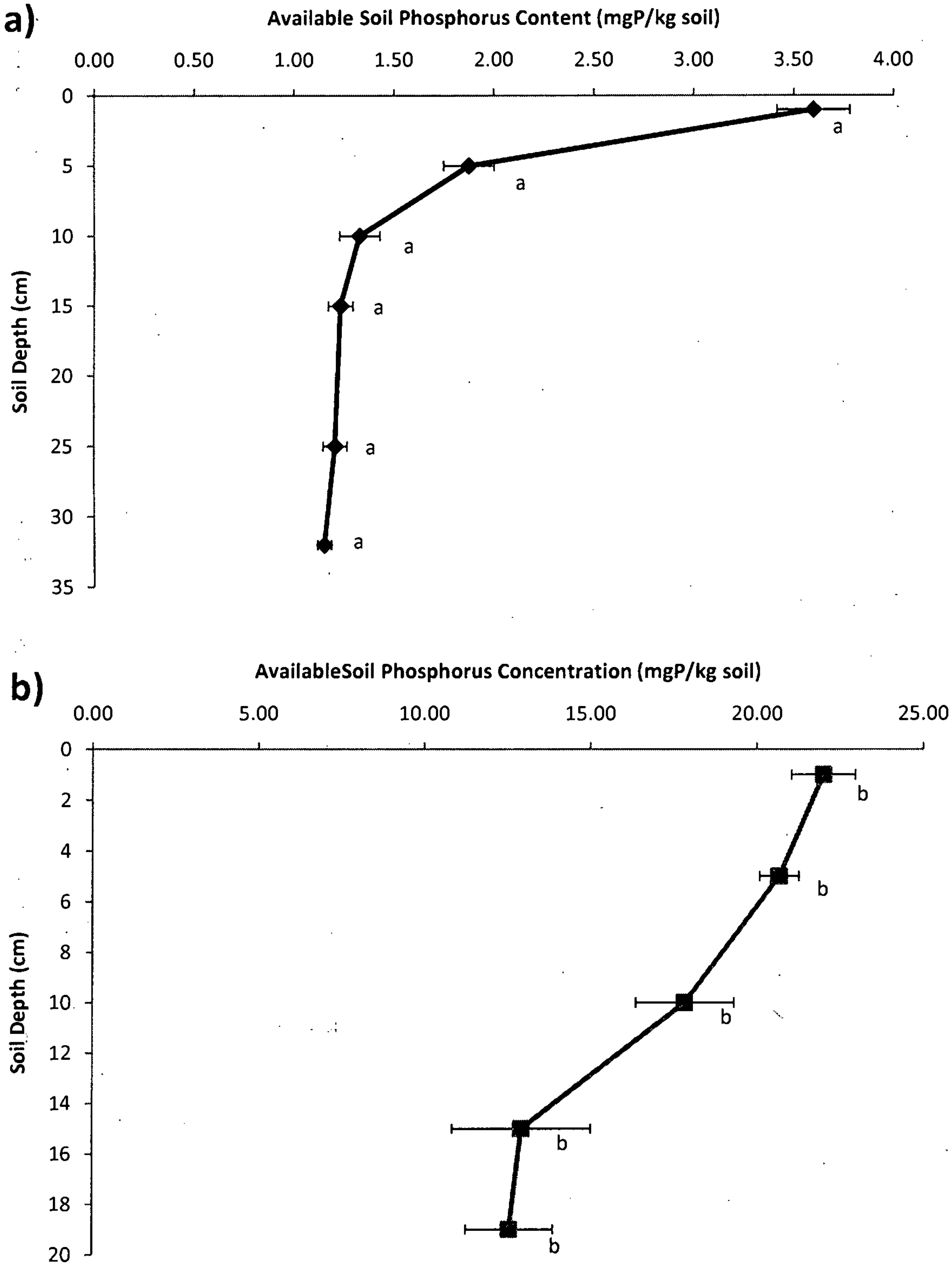

Fig 4:Total available phosphorus in the soil (±SE) a) Pretoriuskop (♦), b) Satara (■).Values are means ± SE. Values with different letters indicate the significant differences ($p<0.001$) between layers and sites.

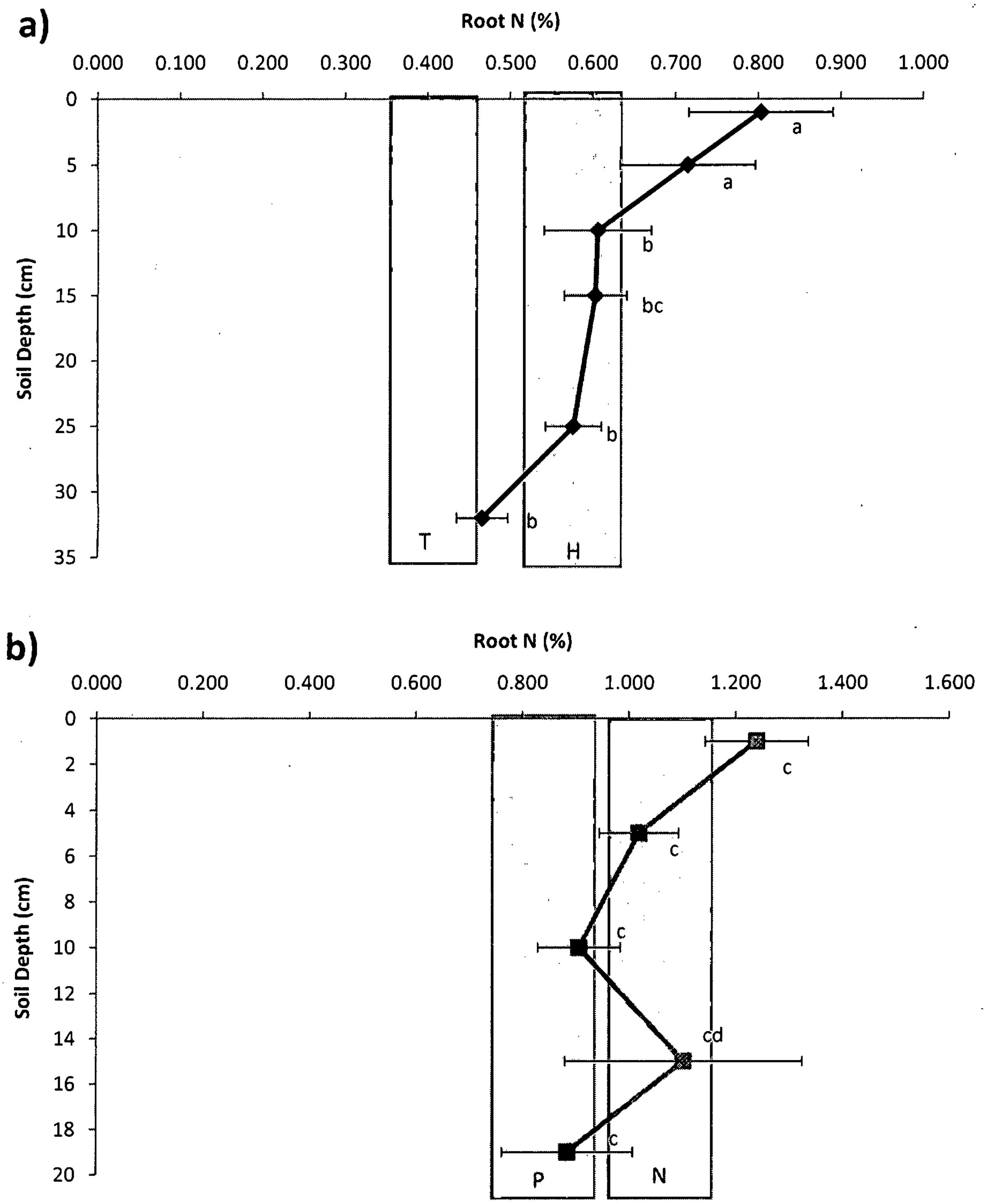

Fig 5: Percentage nitrogen (±SE) for root matter down the soil profile a) Pretoriuskop (♦) and b) Satara (■). The shaded rectangles represent the means ± 1 SE for the end member $\delta^{15}N$ values for *H.filipendula* (H), *T.sericea* (T) and *A.nigrescens* (N) and *P.maximum* (P). Values with different letters indicate the significant differences ($p<0.001$) between layers and sites.

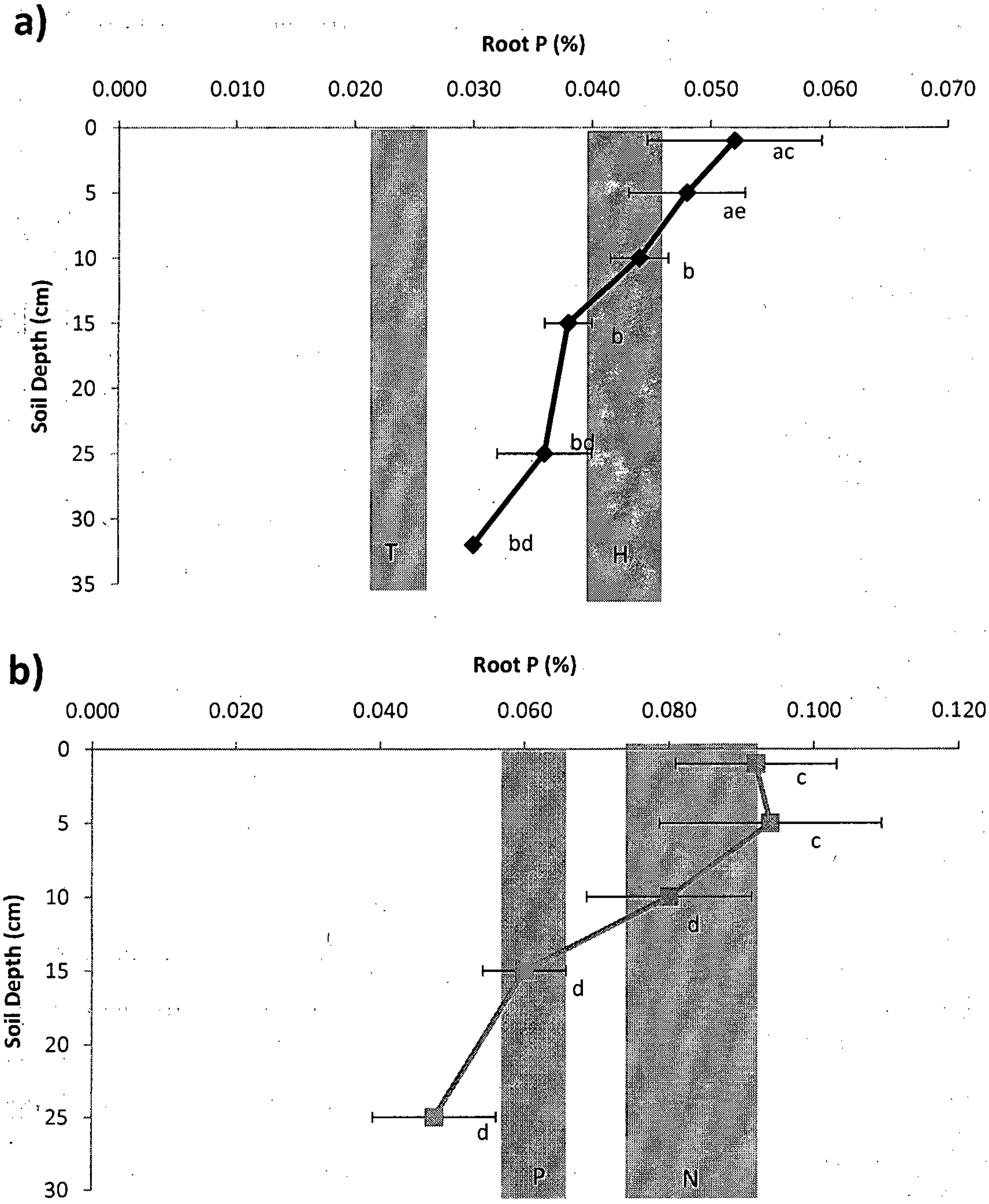

Fig 6: Amount of phosphorus (±SE) in collected root matter down the soil profile in a) Pretoriuskop (♦) and b) Satara (■). The shaded rectangles represent the means ± 1 SE for the end member %P values for *H.filipendula* (H), *T.sericea* (T) and *A.nigrescens* (D) and *P.maximum* (C). Values with different letters indicate the significant differences ($p<0.001$) between layers and sites.