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Research Article

Element concentration in fine roots of woody species in Gelawdios Forest, Amhara Region, Ethiopia

Yibeltal Tigabu Kebede[1*], Boris Rewald[2], Hans Sanden[3], Douglas L. Godbold[4]

[1]Debre Tabor University, Department of forestry, college of Agriculture and Environmental Sciences, Debre Tabor, Ethiopia. Email = yibeltaltigabu@mail.com
[2]Institute of Forest Ecology, Department of Forest and Soil Sciences, Boku University of Natural Resources and Life Sciences, Vienna, Austria. Email = boris.rewald@boku.ac.at/boris.rewal@mendelu.cz
[3]Institute of Forest Ecology, Department of Forest and Soil Sciences, Boku University of Natural Resources and Life Sciences, Vienna, Austria. Email = hans.sanden@boku.ac.at/hans.goeranson@boku.ac.at
[4]Institute of Forest Ecology, Department of Forest and Soil Sciences, Boku University of Natural Resources and Life Sciences, Vienna, Austria. Email = douglas.godbold@boku.ac.at

[*]**Correspondence**
Yibeltal Tigabu Kebede
yibeltaltigabu@mail.com

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Background: Classifying vegetation into functional types is important to improve understanding of ecosystem functioning and to predict the influence of future climate change broadly and specifically. Identifying traits that differ between species and functional types is key to understand species' functioning. Among traits, fine root traits, including chemical traits or concentrations of elements, are fundamental for plant functioning and environmental change response.

Methods: Samples from fine roots of fourteen species repeated six times each, were collected at Gelawdios church forest, Amhara region, Ethiopia. The six sites were separated by 200 meters (180 -200 meters). Chemical composition (concentration of elements) was analysed for the absorptive root of orders 1-3. Eleven elements (N, C, P, K, Ca, Mg, Al, Mn, Na, Fe and S) were analysed using different methods for CN (carbon and nitrogen) and the rest of nine elements.

Results: The studied chemical traits showed significant differences between many species and some functional groups. Carbon and nitrogen showed significant differences between species, but regarding functional groups, only the nitrogen difference was significant (concentrations ranged from 0.65% to 3.21% across species). Significance and non-significance were observed in the other nine elements (P, K, Ca, Mg, Al, Mn, Na, Fe and S) between species and at the same time between functional groups. The concentration values of elements are also highly variable between species.

Conclusion: The results indicated some higher, some similar and some lower values in comparison to global values and other research results. These differences and variations between species could be due to climatic and environmental resource differences at both in micro and macro levels. The study of biochemical root traits was very recommendable to see the extent of its effects on the determination of species' functioning. Although the correlations of these traits were not as strong as the correlations of morphological traits, the difference was wide, indicating that these traits are crucial in functioning and hence in the life strategy of plants. This study has a profound contribution for the understanding of tropical regions' root traits and hence for the improvement of global biogeochemical models.

Keywords: *concentration of elements, root traits, biochemical traits, dry afromontane forest, ecological processes, functional types*

Introduction

Plant functional types bridge the gap between plant physiology and community and ecosystem processes, thus providing a powerful tool in climate change research. Thus, understanding plant functional types and the specific traits that influence ecosystem processes is the first step to understand ecosystem functioning and to tackle potential environmental change problems. Plant functional types are sets of species showing similar responses to the environment and similar effects on ecosystem functioning (Díaz et al., 2007; Sandra & Cabido, 2001). Functional types are determined by set of functional traits. Plant functional traits are any morphological, physiological, biochemical or phenological feature, measurable for individual plants, at the cell to the whole organism level, which potentially affects its fitness for a specific ecosystem process (Garnier et al., 2013). This concept leads to identifying traits that determine species and groups' ecological strategies and responses to the environment. Plant functional traits can be identified on different organisational levels/organs of a plant: leaves, stems, roots, flowers or the whole plant itself. There are a number of contexts in which it is useful to consider functional groups rather than individual species (Englert et al., 2014). The main use is in large scale regional assessments, such as those predicting a plant community's response to climate change or to conservation practices, which benefit from a course, functional group approach' rather than a fine-scale species approach to simulation. Characterizing plant productivity, resistance to disturbances, and management of rare plants are some current uses of plant functional groups approach.

Root traits are among most determinant traits for functional types/groups as they are one of the vital resource acquisition organs. As leaves are the above ground pillar to absorb CO_2, roots are the other end, vital to absorb water and nutrients. This very critically important part of a plant, with smaller share of plant's biomass but considerably higher share of function is not studied to a similar extent as above ground parts. One reason might be the un-visibility of roots as they are below ground, another the methodological difficulties to retrieve samples, and to analyse the required parameters of roots like volume and surface area. However, recently, studies on root traits increased due to an increasing interest of researchers to adaptation strategy of plants to environmental change and to improve our understanding of biogeochemical cycling. As coarse root functions are mainly restricted to anchorage, storage and transport of nutrients and water, major function (nutrient acquisition) and associated carbon costs are associated to fine roots, commonly defined by diameter less than 2 mm.

The traditional definition of fine roots (< 2 mm) is now a days criticised by researchers. There is a tendency that this pool of fine roots has different functions which are nutrient absorption and transport. It is difficult to get consistent data of absorptive roots based on dimeter cut of less than some specific number diameter (Mccormack et al., 2015). Taking less than 1 or 0.5mm diameter for example cannot work due to the variation of absorptive roots depending on species. Some species might have absorptive roots of greater than 1mm diameter and some species might have transport roots less than 0.5mm diameter. Order based classification is becoming more preferable to account this functional difference in more standardised design (Mccormack et al., 2015). Many types of root ordering was tried in the past decades. The developed and recently used ordering is based on stream-order descriptions, where the most distal, unbranched roots are first order and where second-order roots begin at the junctions of two first-order roots and so on (Pregitzer et al., 2002).

Fine roots of order 1-3 are the most nutrient acquisitive part of a root which exhibit wide interspecific variation in morphological and chemical traits (Valverde-barrantes et al., 2015). A question has to be raised "why and how fine roots are the research issue in relation to adaptation to environmental change and biogeochemical modelling?" The answer for this and other related questions resides on the two generalised fine root trait's properties. One of these properties is morphological, chemical and architectural root plasticity. This behaviour of fine roots is quite substantial for plants to adjust their strategy to environmental change and resource deficit challenges. In response to environmental parameters, whole-root systems exhibit high plasticity at different hierarchical scales and forms, such as physiology, anatomy, morphology, and/or biomass (Rewald et al., 2014). The other substantial trait behaviour is its fast turnover rate relative to stem and coarse roots, causing significant C costs (Kawamura et al., 2013).

Basic root traits underlying plant functional types are morphological traits (those related to length, surface area, weight and tips numbers; i.e. parameters related to soil exploration, surface for uptake and associated C costs) and biochemical traits (those related to elements and biochemical compounds).

Biochemical root traits are very crucial in the controlling of ecosystem processes. Traits that determine ecosystem processes such as herbivory resistance, fire resistance, quality timber and growth form and rate are related to plants chemical compound concentrations like elements, lignin, tannins and polyphenols.

We have seen that understanding chemical/elemental root traits and studying their behaviours, differences between species and between functional groups has a tremendous advantage in understanding basically the adaptive strategies of plants to environmental changes and fluxes of carbon for biogeochemical cycle modelling. Root traits play an important role in belowground strategies and fluxes, and root data have become significantly important in the understanding of the above two processes (adaptation strategy and biogeochemical cycling) belowground (Al, 2012). Previous studies on root traits have focused on developed countries and little research has been done on African forest species including those in the remaining fragments of the diverse Ethiopian highland forests. Currently, data on root traits of woody species is predominantly available for temperate and boreal species and information on (fine) root traits on different species and functional groups of dry Afromontane forests in the eastern Africa region is virtually absent. Furthermore, functional types and species are assessed by morphological root traits such as specific root length, specific root area and root tissue densities. However, to achieve an understanding of local ecosystem functioning, to increase the global understanding of forest ecosystems, to provide data for either modelling or management tasks, it is necessary to gather chemical/elemental root trait data of locally important species and functional groups in addition to morphological traits. This study can bring a hint for the root trait research of tropical forests and in comparing results from other regions.

The general objective of the study is to investigate chemical root traits of divergent woody plant species and functional groups in a tropical dry montane forest in the northwestern part of Ethiopia.

(1) We hypothesize that biochemical root traits of woody species differ widely in this ecosystem, and a distinct trait difference exists between evergreen and deciduous plant functional groups.
(2) I further hypothesize that there is a difference between species compared to species of other tropical forest ecosystems and temperate forest ecosystems because of the large seasonal and long time differences in temperature and available environmental resources.

Specific research questions are

- Are there any trait differences between species of the Gelawdios forest ecosystem?
- Are the differences of chemical root traits between 14 woody species larger or smaller than differences in other tropical forest ecosystems and can functional groups of root traits be distinguished according to leaf phenology?
- Does the potential nutrient and carbon input to the ecosystem per unit dry matter of fine roots differ between functional groups?

Materials and methods

Site description

The research site is located in the Amhara region, South Gondar zone, Dera woreda (district). It is at one of the church forests of Ethiopia (Gelawdios church), which acts as a sanctuary for remnant patches of pristine mountainous highland forest previously covering the whole or large area. The forest surrounds a church 55 km northeast of Bahir Dar in the Amhara region (11°38’25” N, 37°48’55” E), NW Ethiopia. It has an elevation range from 2456 - 2526 m ASL wit. The mean annual rainfall is 1216 mm (range 1103–1336), and the average daily temperature is 19.0◦C (range 16.0–23.7) (Sterck, 2008). The main rainfall period is between June and September and a lesser amount of rain is also expected in March and April. Cambisols and Andosols are the predominant soil types in the area (Sterck, 2008; Wassie et al., 2009). The 68 ha forest area is divided by a gravel road and the forest is surrounded by extensively cultivated crop and grassing lands (Figure 1).

Figure 1. The study site “Gelawdios church forest” in the Amhara Region (NW Ethiopia) and the six sampling plots (yellow thick dots) within the forest. The forest map is taken from Google Maps and points are added from GPS readings

Study design

Out of 42 wood species identified(Bongers et al., 2006), we selected fourteen indigenous woody species based on the values of the species importance index considering that they are in different families. Species are selected from the highest importance values up to 16th. We ignored Euphorbia Abyssinia (2nd IVI rank) since it cannot be found in all the six plots. Similarly, we ignored *Eucalyptus globulus* (13th in IVI value) due to its exoticness. So the fourteen species are those IVI rank values of 1-16. The functional groups we are interested in are based on their leaf phenology (Table 1).

Table 1. The selected woody species list including their scientific names, leaf phenology, growth form, heights, height class and families

No.	Scientific name	Leaf phenology	Family
1	*Albizia gummifera*	Deciduous	Fabaceae
2	*Apodytes dimidiate*	Evergreen	Icacinaceae
3	*Bridelia micrantha*	Evergreen	Euphorbiaceae
4	*Calpurnia aurea*	Deciduous	Papilionoideae
5	*Chionanthus mildbraedii*	Evergreen	Oleaceae
6	*Combretum molle*	Deciduous	Combretaceae
7	*Croton macrostachyus*	Deciduous	Euphorbiaceae
8	*Dovyalis abyssinica*	Evergreen	Flacourtiaceae
9	*Ekebergia capansis*	Evergreen	Meliaceae
10	*Maesa lanceolata*	Evergreen	Myrsinaceae
11	*Podocarpus falcatus**	Evergreen	Podocarpaceae
12	*Prunus Africana*	Evergreen	Rosaceae
13	*Schefflera abyssinica*	Deciduous	Araliaceae
14	*Teclea nobilis*	Evergreen	Rufaceae

*Gymnosperm

One randomly selected individual per species was sampled at each of six sampling plots (Figure 1); the distance between sampling plots was >200 meters. That means each tree is sampled six times as a replication. Since the least replication possible in statistics is three, we believed six replications could give us a reliable result. After an individual was selected, four soil monoliths (10 x 10 cm), two at each opposite side of the trunk, were collected to a soil depth of 15 cm for chemical root trait analysis. Very coarse roots were followed from the stem outwards to ensure correct taxonomic identification of sampled tree root segments. Sampled root branches were cut by scissors from the tracked very coarse root (Rewald et al., 2011). After monolith extraction, the soil was removed carefully by hand and the extracted root branches were stored in a water-filled plastic bag. Additional rinsing was conducted in the laboratory within 48 hours after root collection. After washing, the four samples per tree individual were merged into two pairs, combining one sample from each side of the bole: Samples for chemical analysis were immediately oven dried (60°C, 48 hours). All samples were transported to Austria within 4 weeks after sampling. Subsequently, chemical analyses were conducted in the laboratories of the Department of Forest and Soil Sciences and the Institute of Animal Nutrition, Livestock Products, and Nutrition Physiology of BOKU University.

Chemical analyses

For chemical analysis, two samples from opposite sides of each sampled individual were merged as described before. The samples were dried at 65°C for 48 hours to facilitate grinding. A ball mill (Metrohm INULA Gmbh) was used to grind samples by 4 revolutions at 340 rotations per minute for 45 seconds, using a smaller-sized ball mill with smaller-sized balls.

Elemental analysis

Carbon and nitrogen contents were analysed by a nitrogen/carbon analyser (TruSpec CN, Leco, USA). Approximately one hundred mg of ground dry matter sample was used after oven drying for a second time (65°C, 48 hours). Other elements (potassium, sodium, calcium, magnesium, manganese, aluminium, iron, phosphorus and sulphur) were analysed by ICP-OES. Approximately one hundred mg of dry samples were digested by 5 ml of nitric acid solution for 30 minutes with 15 minutes rising time and 15 minutes constant time at 200°C. The digestion was filtered (Whatman filter paper) and diluted in a 50 ml flask. The diluted filtrate was then analysed by an ICP-OES (Optima 8300, Perkin Elmer, USA). The elements were selected based on the facilities of the laboratory of Boku University even though the plan was to analyse the 17 essential elements depending on literature suggestions.

Statistical analysis

We used the free statistical software R to compare traits between species and between functional groups. Replications were number of trees both for species and functional groups. One way ANOVA and Kruskal-Wallis tests were used depending on the type of data. A t-test was used for the comparison of leaf phenology groups. Multiple comparisons were done by Duncan's multiple range test. Linear model and correlation functions were used for the assessment of the relationship between traits and their strength. Normality of data was tested by the Shapiro-Wilk test.

Results

Carbon and nitrogen concentrations

Nitrogen concentrations showed significant differences in the interspecific comparison (Table 2). The minimum nitrogen percentage was observed in absorptive roots of *Maesa lanceolata* (0.65 % by volume). *Combretum molle* and *Chionanthus mildbraedii* have the lowest nitrogen percentage next to Maesa lanceolata. The maximum percentage was observed in *Podocarpus falcatus* (3.21 %). The problem in concluding higher values of nitrogen in gymnosperms than angiosperms is that the only gymnosperm species was *Podocarpus falcatus*. It lacks replicating gymnosperms to get a reliable result. *Bridelia micrantha* and *Ekebergia capensis* also have great nitrogen concentrations similar to *Podocarpus*. The minimum value from all 84 single measurements of trees is found in *Maesa lanceolata* (0.48 %) and the maximum value in *Podocarpus falcatus'* absorptive roots (3.55 %).

Carbon concentration also showed significant differences between species (Table 2). The minimum carbon concentration was observed in absorptive roots of *Teclea nobilis* (47.16 %) and the maximum percentage was observed in *Ekebergia capensis* (53.35 %). *Albezia gummifera*'s and *Calpurnia aurea's* absorptive roots have the next lowest carbon concentrations to the absorptive roots of *Teclea nobilis*.

Table 2. Mean, standard deviation, minimum and maximum values of nitrogen and carbon in volume percentage (Duncan multiple range test, p < 0.01, n = 6). Different letters indicate significant difference between species. All the significances were seen with P-value less than 0.01.

Tree name	N (%)				C (%)			
	Mean	SD	Min	max	Mean	SD	min	Max
Albezia gummifera	1.033 EFG	0.104	0.901	1.143	47.8 DE	0.68	46.97	48.80
Apodytes dimidiata	1.544 CD	0.365	1.065	1.920	49.3 BCD	2.02	47.51	52.23
Bridelia micrantha	2.252 B	0.397	1.904	3.004	49.2 BCD	0.80	47.92	50.30
Calpurnia aurea	1.38 DEF	0.158	1.169	1.562	48.9 CDE	1.00	47.77	50.73
Chionanthus mildbraedii	0.809 B	0.190	0.592	1.096	50.4 BC	0.62	49.56	51.20
Combretum molle	0.809 G	0.190	0.592	1.096	50.4 BC	0.62	49.56	51.20
Croton macrostachyus	1.380 DEF	0.321	0.785	1.740	50.3 BC	0.63	49.54	51.04
Dovyalis abissinica	1.120 DEFG	0.131	0.950	1.293	51.2 B	0.63	50.42	52.16
Ekebergia capansis	1.948 BC	0.269	1.663	2.309	53.3 A	0.45	52.81	53.95
Maesa lanceolata	0.645 G	0.136	0.483	0.815	49.7 BCD	2.02	46.10	51.52
*Podocarpus falcatus**	3.206 A	0.219	2.930	3.554	50.5 BC	0.50	49.85	51.23
Prunus africana	1.503 CDE	0.534	0.748	2.125	49.3 BCD	1.63	47.47	52.16
Schefflera abissinica	0.998 FG	0.132	0.882	1.221	50.5 BC	0.86	49.50	51.68
Teclea nobilis	1.080 DEFG	0.126	0.886	1.223	47.1 E	1.64	44.64	48.89

SD = standard deviation, min = minimum, and max = maximum. *gymnosperm

There were highly significant (p < 0.002) differences in absorptive root nitrogen concentration between groups of leaf phenology. As it is shown in Fig. 2A, the functional group of the evergreen species has a greater nitrogen concentration (1.71 %) than the deciduous species' absorptive roots (1.12 %) (Figure 2B).

The unequal sample size between functional groups has some theoretical impact on the average results of each. A group with a large sample size can give a more representative result than a group with a small sample size. But a sample size of more than 30 can be considered a representative sample. So, it is possible to compare these groups as unequally replicated groups.

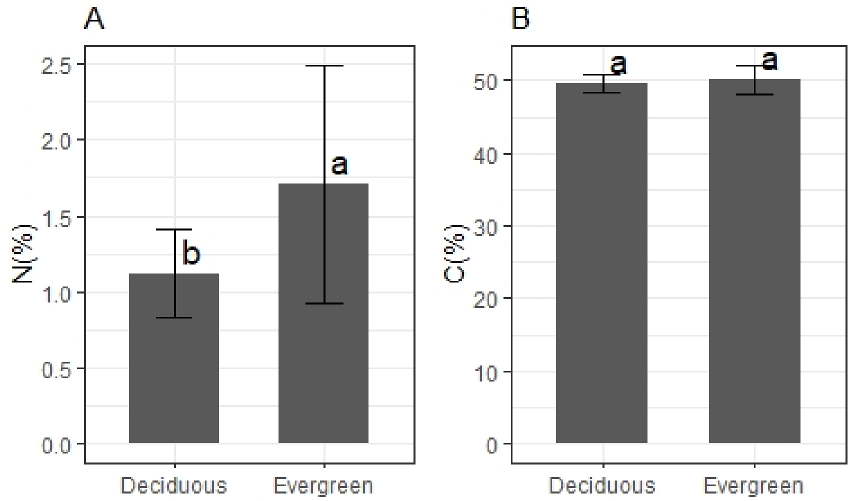
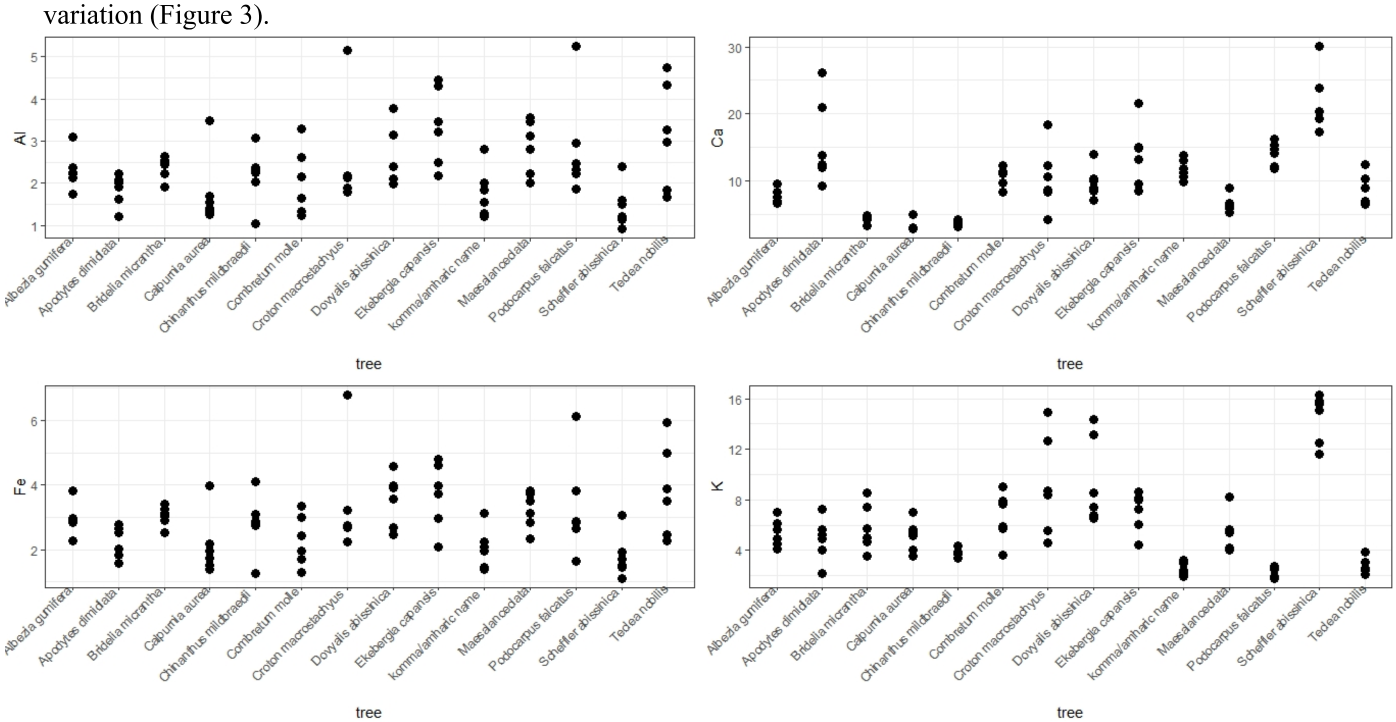


Figure 2. Nitrogen (A) and carbon (B) concentrations in functional groups of leaf phenology (mean ±SE; Duncan multiple range test, $p < 0.01$, n = 30(deciduous), 54(evergreen)) in leaf phenology). Different letters indicate a significant difference between groups. All the significances were seen with a P-value less than 0.01

Other elements

All the other nine analysed elements showed significant differences between species (Table 4) and different patterns were observed (Fig. 3). Frequently, the highest element in absorptive roots is calcium, except for *Calpurnia aurea* and *Bridelia micrantha*. For these two species, potassium is the element with the highest concentration. Potassium has the second-highest concentration in most of species. Manganese has the lowest concentration in the absorptive roots of all fourteen species. The coefficient of variation of elements was checked across species and within individuals of a species. Different variations were seen in both individuals and across species. Aluminium, iron, phosphorus and manganese showed less variation between species, whereas magnesium, calcium and potassium showed higher variation (Figure 3).



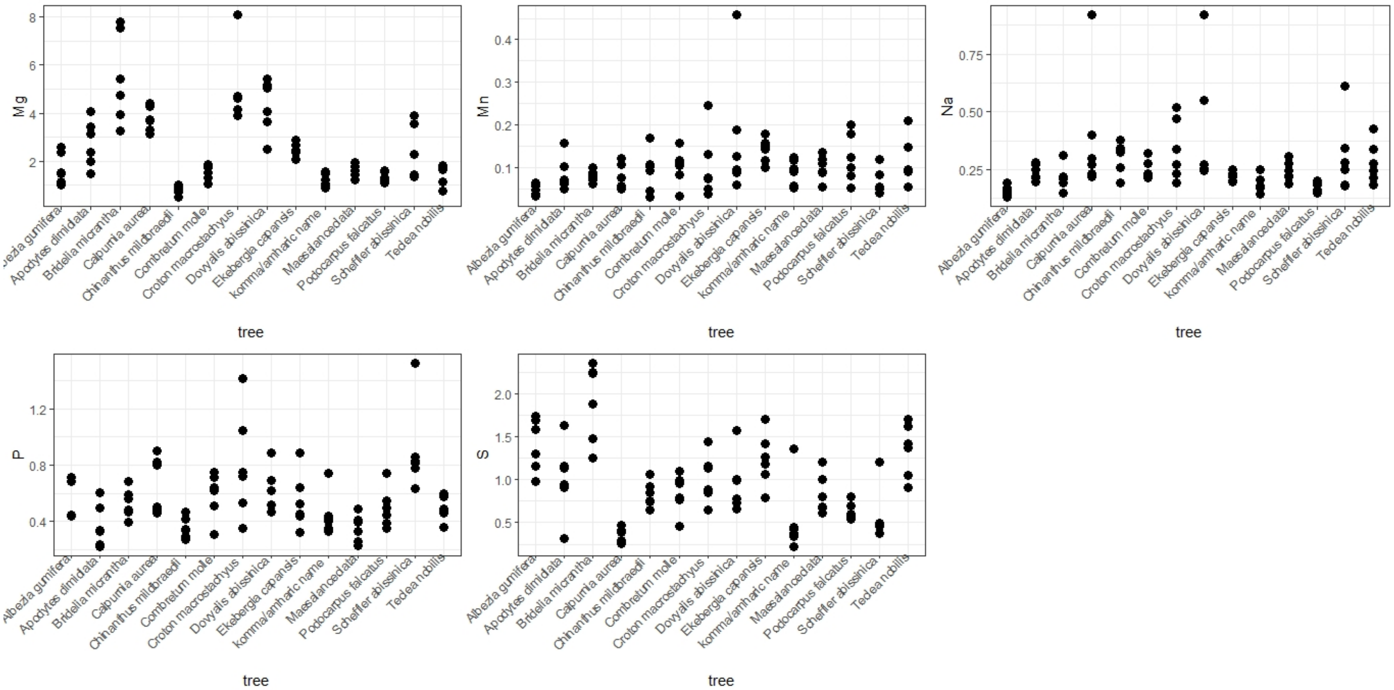


Figure 3. Distribution of the nine elements within the six individuals and in comparison, to the fourteen species. Some species do not show six dots due to the overlapping of closer values and the condensation of the graphics. Zooming in helps a little

The lowest sulphur concentration was observed on *Calpurnia aurea* and *Prunus africana*. *Schefflera abissinica* has also a lower sulphur amount. *Bridelia micrantha* has the highest sulphur concentration of all species. *Ekebergia Capensis, Teclea nobilis*, and *Albezia gummifera* have relatively higher sulphur concentration. *Ekebergia capensis* has the highest aluminium concentration. *Schefflera abissinica* and *Calpurnia aurea* have the lowest aluminium concentration. Calcium is one that varied most between species from a minimum concentration of 3.68 mg/g (*Calpurnia aurea*) to a maximum concentration of 21.38 mg/g (*Schefflera abissinica*). *Chionanthus mildbraedii* and *Podocarpus falcatus* are the species of second smallest and next higher amounts of calcium, respectively. Iron has a variation between a minimum of 1.75 mg/g (*Calpurnia area*) and 3.84 mg/g (*Teclea nobilis*). *Ekebergia capensis* and *Dovyalis abissinica* are from species of high iron concentration. Potassium is minimum in *Podocarpus falcatus* and maximum in *Schefflera abissinica*. *Teclea nobilis Prunus africana* and *Chionanthus mildbraedii* have also very low potassium concentration. But *Ekebergia capensis, Dovyalis abissinica* and *Croton macrostachyus* have relatively higher potassium concentration. Magnesium has minimum concentration of 0.79 mg/g in *Chionanthus mildbraedii* and maximum concentration of 5.45 mg/g in *Bridelia micrantha. Croton macrostachyus* and *Dovyalis abissinica* also showed higher magnesium concentration. Manganese showed almost similar concentration in all species with 0.09 mg/g difference between maximum and minimum concentration (maximum and minimum are 0.14 and 0.049 mg/g respectively, in *Ekebergia capensis* and *Albezia gumifera*). Sodium is the other less varied element between species. Its maximum concentration is in *Dovyalis abissinica* (0.41 mg/g) and its minimum concentration is found in *Albezia gummifera* (0.155 mg/g). *Prunus africana* and *Podocarpus falcatus* are species of lower sodium concentration whereas *Chionanthus mildbraedii*, *Schefflera abissinica, Calpurnia aurea* and *Croton macrostachyus* have relatively higher sodium concentration. Phosphorus varied between 0.35 mg/g (*Chionanthus mildbraedii* & *Maesa lanceolata*) and 0.905 mg/g (*Schefflera abissinica*). *Combretum molle* also showed higher phosphorus concentration. *Apodytes dimidiata* and *Prunus africana* showed lower concentrations of phosphorus.

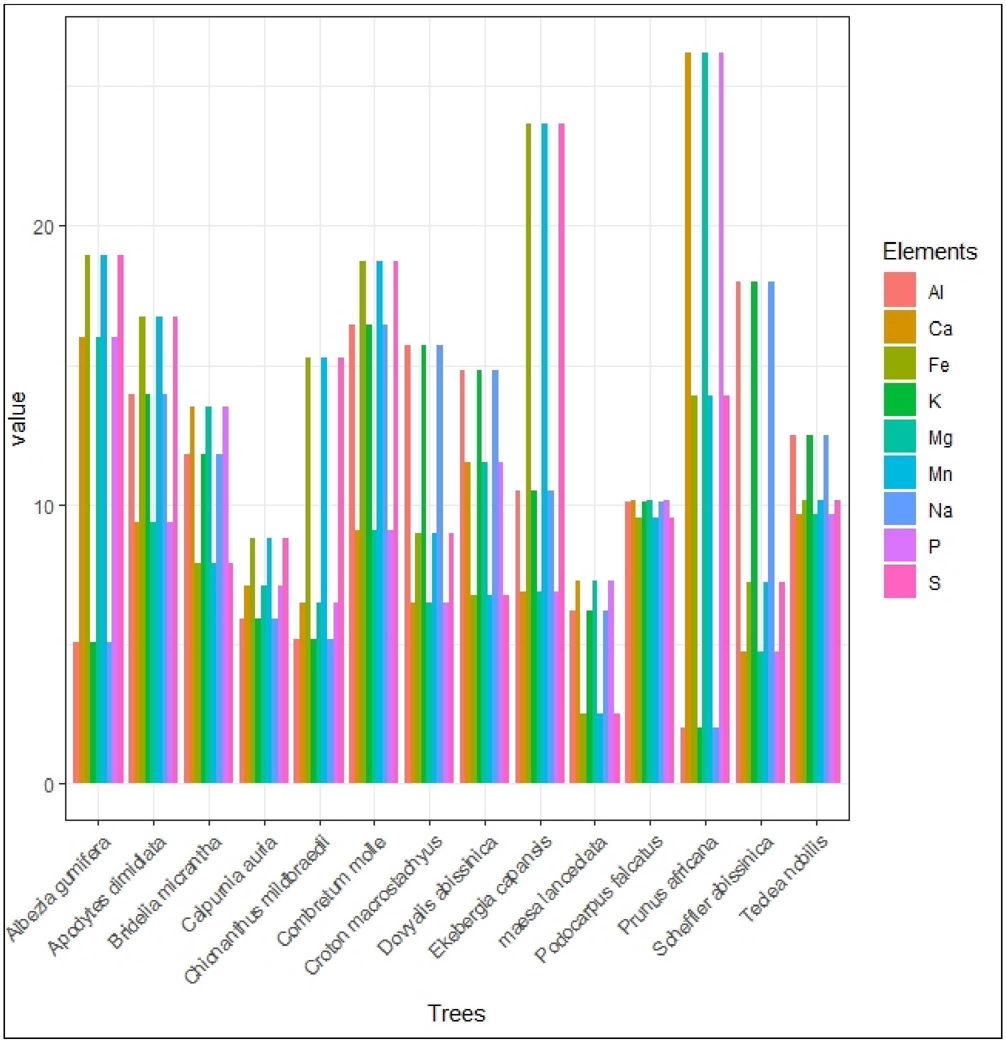


Figure 4. Visualization of patterns and quantity of the nine elements S, Al, Ca, Fe, K, Mg, Mn, Na, and P across 14 species and within individual woody species from the Galowdios church forest in NW Ethiopia

Table 3. **Some of the nutrient ratios that have substantial influences in the ecosystem process**

Species	C:N	C:P	N:P	Ca:Al
Albezia gumifera	46.3	90.85	3.55	1.96
Apodytes dimidiata	31.98	119.56	8.59	3.73
Bridelia micrantha	21.88	93.17	1.88	4.25
Calpurnia auria	35.46	74.14	2.53	2.09
Chionanthus mild-braedii	62.4	143.4	1.76	2.29
Combretum molle	62.41	85.72	5.11	1.37
Croton macrostachyus	36.47	62.76	5.12	1.72
Dovyalis abissinica	45.79	84.62	3.42	1.84
Ekebergia capansis	27.3	98.43	4.12	3.59
maesalanceolata	76.9	141.59	2.32	1.83
*Podocarpus falcatus**	15.75	103.1	4.94	6.54
Prunus africana	32.82	110.35	6.6	3.36
Scheffler abissinica	50.65	55.85	14.6	1.1
Teclea nobilis	43.6	96.24	2.88	2.2

*gymnosperm

The ratios of elements C: N, C: P, N: P and Ca: Al were calculated among the elements (Table 5). C: N ratio varied between 15 (*Podocarpus falcatus*) to 77 (*Maesa lanceolata*). *Chionathus mildbraedii* and *Combretum molle* showed a higher C: N ratio next to *Maesa lanceolata*. C: P ratio is the highest ratio of all four ratios. It ranges between 55.8 (*Schefflera abissinica*) to 143.4 (*Chionanthus mildbraedii*).

Table 4. Mean values of the nine elements of absorptive roots in all species. Different letters indicate statistically significant differences between means (Duncan multiple range test, p < 0.01, n = 6)

Species	S (mg/g)	Al (mg/g)	Ca (mg/g)	Fe (mg/g)	K (mg/g)	Mg (mg/g)	Mn (mg/g)	Na (mg/g)	P (mg/g)
Albezia gummifera	1.41 B	2.30 ABC	8.17 EFGH	3.09 ABC	5.40 CDE	1.69 DE	0.049 B	0.15 C	0.527 BC
Apodytes dimidiata	0.92 BCDE	1.78 BC	15.30 B	2.06 BC	5.43 CD	2.65 CD	0.093 AB	0.25 ABC	0.413 C
Bridelia micrantha	1.91 A	2.37 ABC	4.38 GH	3.03 ABC	5.82 CD	5.45 A	0.079 AB	0.21 ABC	0.529 BC
Calpurnia aurea	0.36 E	1.45 BC	3.68 H	1.75 ABC	5.13 CDE	3.76 BC	0.076 AB	0.39 AB	0.660 ABC
Chionanthus mildbraedii	0.84 CDE	2.18 ABC	3.84 H	2.82 ABC	3.81 CDE	0.79 E	0.090 AB	0.30 ABC	0.352 C
Combretum molle	0.84 CDE	2.04 ABC	10.44 CDEF	2.29 ABC	6.63 BC	1.55 DE	0.099 AB	0.25 ABC	0.589 BC
Croton macrostachyus	1.02 BCD	2.03 ABC	10.41 EFG	2.72 ABC	9.13 B	5.02 AB	0.101 AB	0.34 ABC	0.802 AB
Dovyalis abissinica	0.96 BCD	2.87 AB	9.83 CDEF	3.54 AB	9.46 B	4.29 AB	0.169 A	0.41 A	0.606 ABC
Ekebergia Capansis	1.24 BC	3.35 A	13.82 BC	3.69 AB	7.09 BC	2.48 CD	0.140 AB	0.22 ABC	0.542 BC
Maesa lanceolata	0.83 CDE	2.87 AB	6.68 FGH	3.22 ABC	5.27 CDE	1.53 DE	0.098 AB	0.25 ABC	0.351 C
*Podocarpus falcatus**	0.63 DE	2.85 AB	14.01 BC	3.32 ABC	2.2 E	1.33 DE	0.122 AB	0.17 BC	0.490 BC
Prunus africana	0.36 DE	1.78 BC	11.76 BCDE	2.04 BC	2.47 E	1.19 DE	0.088 AB	0.18 BC	0.447 C
Schefflera abissinica	0.58 DE	1.46 C	21.38 A	1.79 C	14.40 A	2.31 D	0.071 AB	0.31 ABC	0.905 A
Teclea nobilis	1.35 BC	3.14 AB	9.05 DEFG	3.84 A	2.76 DE	1.39 DE	0.115 AB	0.28ABC	0.490 BC

*gymnosperm

Groups of leaf phenology (deciduous/evergreen) have significant differences in Aluminium, potassium, manganese, phosphorus and iron concentrations in absorptive roots (Fig. 10A-E). Evergreen species have higher Al, Mn and Fe and lower K and P concentrations than deciduous species. Other element's differences are insignificant between leaf phenology groups.

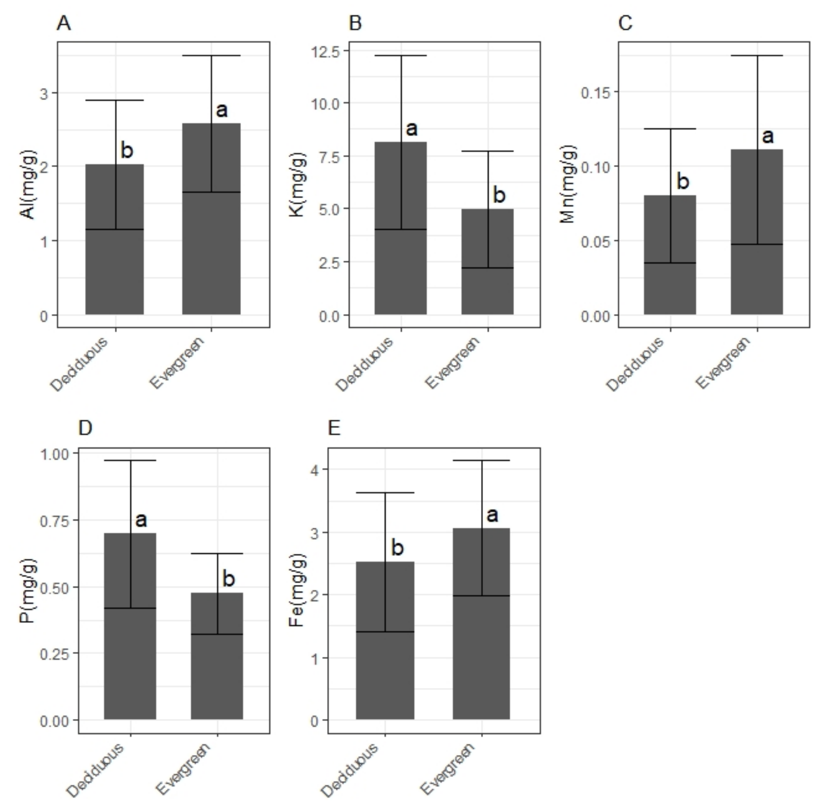


Figure 5. The mean values of the elements Al, K, Mn, P, Fe between functional groups of leaf phenology (mean ± SE; Duncan multiple range test, p < 0.01, n = 30(deciduous), 54(evergreen)). Different letters indicate significant difference between groups

Discussion

Trait differences of absorptive roots between species and relation to functional groups

Carbon and nitrogen concentrations of absorptive roots in our study showed significant differences between species. The nitrogen concentration ranges from 0.64 % to 2.252 % between angiosperm species (13 species). The single gymnosperm species (*Podocarpus falcatus*) showed a higher amount (3.206 %), but since it does not have enough amount of replication (we don't have some extra gymnosperm trees), we cannot take it as a reliable estimate of higher concentration for gymnosperms. The average nitrogen concentration for the fourteen species is 1.498 % which is in a very wide range of many research results. Among these to mention is: 1.11 % in the works of Gordon and Jackson (2000), synthesized from a large database of fine root nutrient content research, 1.71% in (Jackson et al., 1997) both works include different biomes, climates and vegetation types, a range of 1.26 – 1.39 % between different stand types, in (Lugli et al., 2024), 1.55 % in (Xia et al., 2015) and a range of 1.39 -2.09 % between different species in (Russell, 2014). Very recent paper with similar findings was 1.50 % in the degraded areas of the Peruvian Amazon (Dezzeo et al., 2021). Another important recent finding of a research, done in the Sal-dominated forest ecosystems of Central Himalaya, India, on different sites and species for two years, showed a range of 1.10 – 1.71, its mean comparable to our results (Pandey et al., 2023). In the literature reviewing process, some deviated results are found. Results as equal as our least result (0.64%) was for example seen in the research results of (Ludovici & Kress, 2006). Also, an average of 0.74 % nitrogen concentration was found in Lugli's work (Lugli et al., 2021). Ludvici & Kress did the research in the regions of North America, similar in climate to our research region, but Lugli and others did in a highly tropical region of Brazil, having much higher annual rainfall and temperature than our research region. A very considerable large result of nitrogen concentration was found in the research of (Terzaghi et al., 2016) which ranged from 7.65 g/kg to 9.72 g/kg. The unit in our data analysis was in mg/gm and in conversion to g/kg, numbers do not change; that means, 7.65 g/kg = 7.65 mg/g. The region is, of course, believed to bring a difference since it is the European alpine region which can be snow-covered for a season.(Terzaghi et al., 2016).

Our carbon result ranged between 44.67 % and 53.23 % with an average value of 49.94 % for the fourteen trees. This result is not far from the commonly expected carbon concentration (50%) of plant tissues (Westlake, 1966, as cited by Gibbs *et al.*, 2012). Many results also showed almost similar means and ranges of results, for example 48% in (Gordon and Jackson, 2000), 48.8 % in (Jackson, Mooney and Schulze, 1997) and a range of 42.8-49.9 % in (Socher et al., 2013). A relatively lower result was reviewed, for example, 42.55 % (Hu et al., 2023), 43.82 % in (Lugli et al., 2021), 45.4 %, in (Ludovici & Kress, 2006), a range of 44.26 – 46 % in (Zhu et al., 2021) and 42.8 -47.3 in (Russell, 2014). A very low result of carbon (31.92 %) was fond in the recent study of (Pandey et al., 2023) on the Central Himalaya. The study site is described as having highly variable weather elements (0-576.5 mm rainfall and 4 - 40°C temperature. This indicates that the area is arid and degraded, so that lower carbon content was found. But degraded areas showed higher nitrogen concentrations (Dezzeo et al., 2021; Pandey et al., 2023). Higher results than our finding was also seen. For example, (Terzaghi et al., 2016) found a range of 52.1 -54.4 mean values in different stands of European beech (*Fagus sylvatica* L.) in the Italian alps. What findings in different regions and years showed is that nutrient contents vary depending on climate, soil types, stand types, although there are some figures put as global results. Most results, of course much with those global results, but too extreme results are also found due to the mentioned factors. The results of our research on all the elements are under this rule.

Some ratios of elements in the tissues of plants have implication on the plants' functional strategy and environmental resources and stresses. Most influential nutrient ratio is C:N. But there are also researches about the importance of ratios of C:P, N:P and Ca:Al. So we tried to examine these ratios. The global fine root expected C:N ratio is 42 (Jackson et al., 1997; Mccormack et al., 2015). We have got almost a similar average C:N ratio result of 42.15 with a variation of a minimum 15.8 in *Podocarpus falcatus* to a maximum 77 in *Maesa lanceolata* for the fourteen species. There are many findings with exactly equal or closer to these global results. But also, there are research results considerably lower and higher results of C:N values than our result. To mention some findings of lower results, a range of 22.8 - 32.6 C:N value was found between fine roots of six tropical tree species (Valverde-barrantes et al., 2015). An other lower result was in the sugar maple forest community of America which is 34.4 (Xia et al., 2015). Higer values of C:N ratios are also found in two species, *Ilex pedunculosa* = 51.5, *Qurecus serrata* = 61 for fine roots of 0.5-2 mm, for roots of < 0.5 mm, they all have lesser values of C:N (Kawamura et al., 2013). Another result on pine (*Pinus taedata*) showed 0.41% N and 45.8 % C with C:N ratio of 111.7 (Ludovici & Kress, 2006). The carbon concentration of pine in this study is in the range of our results and expected carbon concentration of plant tissue. But due to lower nitrogen concentration, it has got higher C:N ratio than both this study's result and global fine root C:N ratio. Result of research on European beach showed 0.85 % nitrogen concentration and 53.6 % carbon concentration which gives C:N ratio of

62.8 (Terzaghi et al., 2013). It falls under the range of our results. Recent findings of (Dezzeo et al., 2021) showed a variations of very lower value(32.08 %) in the highly degraded area to higher value(62.01 %) in the undegraded areas.

We found mean C:P ratio 97.13 of the fourteen trees, varied with minimum of 55.86 in *Sheflera abbysinica* and maximum of 143.45 in *Chionanthus mildbradii.* Most results in the literature showed a very larger values of C:P ratio than our result. For example (Gordon and Jackson, 2000) showed C:N:P ratio 522:12:1 indicating C:P value = 522. An other similar higher value was in (Jackson et al., 1997) which is 450:11:1 C:N:P ration indicating C:P ratio = 540. In (Lugli et al., 2024), they found C:P values of 1210 and 1301 on the fine roots of tropical forests in the two research sites of Guiana. The elemental analysis value of (Jackson et al., 1997) which gave 48.8 % C and 0.11% N also gives higher value of 443.64. Although the majority of research findings gave such a large result in comparison to our result, some values are also found in smaller fashion. For example, in (Ludovici & Kress, 2006), 45.4 % C and 0.78 % N, gives 58.2 and in (Lugli et al., 2021) a value of 95.26 can be calculated from vales of 43.82 % C and 0.46 % N.

N:P value of our result showed a mean of 2.71 with variation from 1.1 minimum to 6.54 maximum values. Relative to all other three ratio values N: P value do not vary widely. The N:P values seen from the references cited above, showed a higher results ranging from 11 (Jackson et al., 1997) to 37.19 in (Lugli et al., 2024) and a lower result ranged from 0.81(Ludovici & Kress, 2006) to 1.6.(Lugli et al., 2020). The global fine root N:P ratio is 11.5 (Zhu et al., 2021). Results of (Li et al., 2023) showed the three ratios the C:N, C:P, and N:P ratios 48.87, 941.77, and 19.80, respectively.
The next considered nutrient ratio to be examined was Ca:Al. It gives a mean value of 4.82 with variations of minimum of 1.76 and a maximum of 14.64. Most literatures do not consider this ratio. But we see the element analysis values of some research. Al is not much researched element. In (Smith, 1995), Ca is 65 mimol/g and Al is 27 mimol/g, giving Ca:Al value of 2.4. In (Zhao et al., 2023), Al values were found ranging from 1.16 to 3.56 and Ca was found ranging from 7.54 to 12.53, giving Ca:Al values ranging from 3.52 to 6.5 in 10 different tree species. In (Akburak, 2020), Al was found from 8.8 to 12.4 and Ca was from 4.77 to 7.34, giving values of 0.54 to .58 in four different tree species. Units fro Ca and Al are in g/kg in both sources (Zhao & Akburak). The compiled results of many experiments to see Ca:Al as indicator of Al and acidity stress on different species, indicated a range of 1.6 -17.32 in fine roots of controls (Vanguelova et al., 2007).

The other nine elements also showed a large variation between species. The concentrations of P, K, Mg and in pine (*Pinus taedata*) fine roots in Scotland, North Carolina USA are similar to our result (Ludovici & Kress, 2006). However, Ca is much smaller (3.35 mg/g) relative to our result which has maximum value of 25 mg/g. Results of a study on red spruce (*Picea rubens*) also showed much lower Ca (65µg/g) than our result (Smith, 1995). The high concentration of Ca in our study is likely due to high Ca availability in the tropical soil compared to the likely more acid pine and spruce forest soils. But concentrations of Mg, Al and Fe in fine roots of red spruce were similar to our result (Smith, Shortle nad Ostrofsky, 1995). We found relatively higher values of sulphur (S) from results of (Smith, 1995; Jackson, Mooney, 1997; Zhao et al., 2023). A good amount of elements (12 elements excluding C and N) were analysed by (Akburak, 2020). All our elements (S is not analysed) showed on average higher values may be due to Mediterranean climate with lower temperature and rainfall in the Akburak's study area (Turky, Istanbul).

The nitrogen concentration in the absorptive roots showed high significant difference between functional groups whereas carbon concentration did not show significant difference between functional groups. The insignificance of carbon supports that plant tissue has on average the same amount of carbon content. Research results also support this result(*Carbon Content between…*, n.d.) although deciduous species can use carbon more efficiently(Luo et al., 2024).
The evergreen species showed higher nitrogen than deciduous species could be due to difference in some specific functioning. But no literature can sport this result. Many of the findings showed higher nitrogen in deciduous species than in evergreen species (Ellsworth & Sternberg, 2016; Mueller et al., 2012; Takashima et al., 2004; Thurner et al., 2025). So this finding needs more research to support it and to justify the cause or to conclude as a mistake.

As seen in the result section, some elements were significant and some are not between functional groups of leaf phenology. To compare the results of elements by leaf phenology is not easy as there is no research which studied these functional groups elementwise.

Nutrient influxes to the ecosystem processes

Quantifying nutrient influxes to the soil system especially carbon influx is one of the current interest in the study of biogeochemical cycling and modelling (Jackson et al., 1997). Nutrient influxes to the soil system per annum from fine roots is generally dependent on rates of biomass production. However, when viewed in detail, a lot of factors can contribute to quantifying nutrient influx in addition to biomass production. Rate of decomposition and concentration of nutrients are the other important factors. Due to the type of nutrients and chemical compounds (Xia et al., 2015) as well as the physical protection to decomposition in thick roots, longevity of fine roots showed a negative correlation to root diameter (John et al., 2000). This differs between functional groups for example between woody species and others (John et al., 2000). We could better predict the potential nutrient input to the forest soil by taking the root concentration of nutrients and compounds into account.

Our data shows that *Schefflera abissinica* has higher concentration of many elements and it has the highest cumulative elements concentration (Figure 3 and Table 4). This indicates that this specie plays an important role in the elements biogeochemical cycling by adding relatively high amounts of nutrients to the soil per unit biomass decomposed. *Ekebergia capensis, Dovyalis abissinica, Croton macrostachyus* and *podocarpus falcatus* also have high element concentrations. *Calpurnia aurea* and *Chionanthus mildbraedii* on the other hand are the species with the lowest concentration and in turn lowest cumulative element concentration. Other species with low element concentrations are *Prunus africana, Maesa lanceolata, Teclea nobils*, *Combretum molle*, *C.aurea* and *C.mildbraedii*. This condition leads to the conclusion that these species have smaller recycling rate of elements independent of decomposition rate. The nutrient and carbon input to the soil is however not only dependent on concentration but also on decomposition rate. The decomposition rate of roots depends on the composition of the other main components, cellulose, hemicellulose and lignin, the concentration of phenols and tannins (Xia et al., 2015). All the findings cited above in the other topics can be taken as a comparison discussion on the influx rates.

Conclusion

The results of the study confirmed that we can conclude on the different hypothesis we proposed about root traits of species and functional groups. The chemical traits, that are, element concentrations, carbon and nitrogen concentration and some of their ratios are significantly different between species. Similarly, they showed differences between functional groups. So, the conclusion is that root traits are different between species depending on size, growth form, leaf phenology and other ecological processes of species. The result of nitrogen between functional groups gives oppositely contrasted result from pervious results. So, our result should be checked and should be confirmed or rejected in terms of this specific result. The nutrients and chemical compounds fluxed to the forest soil per dry mater of plant root tissue is different between species and between functional groups. In our fourteen species, we found that cumulative element influx is higher in the species of *Shefflera abissinica, Croton macrostachyus, Ekebergia capensis* and *Dovyalis abissinica. Calpuria aura* and *Chionanthus mildbraedii* have lower cumulative element influx to the soil. Functional types have also different influx rates in some of the nutrients. In general, the results of the study are important information about chemical root traits of species and functional groups of dry Afromontane vegetation of Gelawdios church forest. Results have some lower, some similar and some higher trait values when compared with previous studies. The general recommendation depending on this study is that additional study on other species and functional groups is necessary for better understanding of role of root traits on determination of ecological processes through determination of functional types. Different traits should be addressed in the identification of their importance on determination of species and functional groups. There is ample amount of both morphological and chemical root traits. Root architecture for example has considerable role in the plant's nutrient acquisition strategy. So, studies in the root structure and root anatomy should be addressed. Other chemical traits such as proteins, waxes, and lipids should also be studied in order that it will be possible to see if they can explain species and functional groups more detail.

Limitations

The study has some considerable limitation than can be taken for future research projects on root traits. The first limitation is that the sampling design might not be reliable enough. We assumed trees can be taken in the forest by some enough number of replications. So, we don't repeat trees in every sampling plot. The second limitation was that the analyzer machine in the laboratory of Boku University cannot able to analyze all the essential elements. So, it was not possible to analyses all the 17 essential plant nutrients.

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Author contributions

Yibeltal Tigabu designed the proposal, collected the data, processed and analysed the data, wrote the manuscript. Priv.-Doz. Dipl.-Biol. Dr. Boris Rewald, Dipl.-Biol. Dr. Hans Sanden and Univ. Prof. Ph.D. Dr. Douglas L. Godbold advised in designing data collection, data processing and analysis and manuscript writing

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Conflict of interest

The authors declare no conflict of interest.

Ethics approval

Not applicable.

AI tool usage declaration

The authors did not use any AI and related tools to write this manuscript.

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