Nitrogen

Article

Changes in Ammonium-to-Nitrate Ratio along *Faidherbia albida* Tree Age Gradients in *Arenosols*

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Abstract: *Faidherbia albida* can enhance the bio-physicochemical fertility of inherently infertile *Arenosols*. Changes in the soil ammonium (NH_4^+)-to-nitrate (NO_3^-) ratio have agricultural, environmental, and ecological implications. Thus, the present study mainly examined the changes in *Arenosol* NH_4^+/NO_3^- ratios, as influenced by varying *Faidherbia albida* tree age classes. We collected 40 composite soil samples (4 tree age classes × 2 soil depths × 5 replications) from 0 to 15 and 15 to 30 cm soil depths following core and auger sampling techniques. Analyses of variance have shown that the highest NH_4^+/NO_3^- ratios for soil under the old tree canopies are followed by the medium age. In contrast, the significantly lowest NH_4^+/NO_3^- ratios were recorded for soil out-of-canopy. Pearson correlation analysis revealed that the NH_4^+/NO_3^- ratio was strongly and positively correlated with clay content, total nematode abundance, the fungi/bacterial biomass ratio, cation exchange capacity, microbial biomass carbon, total nitrogen, and soil organic carbon but negative for sand content, bulk density, and pH. The increment in the plant-available forms of nitrogen might be attributed to the accumulation of biologically fixed nitrogen by the *Faidherbia albida* tree–*Rhizobium* bacteria symbiosis. The NH_4^+/NO_3^- ratio approached one in soil under the old *Faidherbia albida* trees. The findings suggest that (1) the ability of soil to retain NH_4^+ increased and (2) the rate of nitrification might be decreased due to inhibition of nitrification by direct and indirect effects of the tree on the nitrifying group of bacteria. The increment in the NH_4^+/NO_3^- ratio could also be due to the lowering of the soil's pH in the older *Faidherbia albida* trees because lower pH is known to inhibit the activities of nitrifying bacteria. Moreover, maintaining older *Faidherbia albida* trees in farmlands could contribute to retaining nitrogen and trigger below- and above-ground communities' successions and ultimately surpass the productivity of arid and semi-arid *Arenosols*.

Keywords: ammonium retention; *Arenosols*; nitrification; reversed phenology

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1. Introduction

Nitrogen is one of the most important determinants of ecological processes and functions [1]. It is among the most essential of plant mineral nutrients. A large amount of available nitrogen is required to support plant growth and boost crop productivity [2].

NH_4^+ and NO_3^- are the major plant-available forms of nitrogen that can be absorbed and utilized by plants [3]. The simultaneous availability and/or application of both NH_4^+ and NO_3^- are recognized to better increase crop yield and quality [4]. High and low levels of these nitrogen forms in natural and agricultural ecosystems have production, environmental, and or ecological implications [5]. Because their low levels limit land productivity [6], their loss from terrestrial ecosystems, particularly nitrate, causes environmental consequences [7], and their proportion also affects both productivity and biodiversity [8] because these forms of nitrogen have contrasting effects on different plant species and microbial communities [9].

Some studies have revealed that excessive amounts of NH_4^+ in soil may induce toxicity in plants [10], and other research has shown that the application of the NH_4^+ and NO_3^- nitrogen forms was found to increase cereal crop yields compared with NO_3^- alone [11]. Chen et al. [12] also reported that the NH_4^+/NO_3^- ratio of 75:25 significantly increased soluble sugars, starch, soluble protein, and soluble phenols in *Carya illinoinensis*. Therefore, although toxicity is usually observed in many plant species when a large amount of NH_4^+ is provided alone, co-provision with nitrate increases growth performance that can exceed maximal growth rates compared to the sole application of plant-available nitrogen forms by as much as 40 to 70% [10]. Evidence by Subbarao [13] showed that some varieties of the same crop species respond better to ammonium than others, which suggests that crop selection and breeding can enhance ammonium yield benefits.

On the other hand, when plants take up nitrogen in the form of NO_3^-, it must be reduced to amine groups within the plant tissues, and this is an energy-consuming process, whereas the energy consumption in the absorption and assimilation of NH_4^+ is lower than that of NO_3^- [14]. Therefore, the growth and abundance of some plants in a given ecosystem may be explained by their response to either or both of the plant-available forms of N [15,16].

In the soil ecosystem, anaerobic conditions and low pH may hinder the nitrification process by affecting the activities of nitrifying bacteria. In such cases, the plant-available forms of nitrogen become only or predominantly NH_4^+ [17]. In humid tropical and subtropical forest acidic soils, ammonification is highly favored compared to nitrification, subsequently resulting in a greater proportion of NH_4^+ compared to NO_3^- [18].

Tree species can influence the abundances of ammonium-oxidizing functional groups of bacteria, and in certain circumstances, plant species directly affect the abundance of nitro-bacteria due to biological nitrification inhibition [19,20]. Moreover, plants are directly or indirectly known to inhibit [21] or stimulate nitrification [22]; that is, the microbial-mediated conversion of NH_4^+ to NO_3^-, and these processes alter the proportion of the two available forms of nitrogen. Current models reveal that nitrification inhibition primarily induced by plants could significantly impact ecosystem functioning and lead to increased primary productivity and the sustainability of soil fertility [23].

Most importantly, the inhibition of nitrification enhances the retaining of mineral N in soil ecosystems as NO_3^- is more leachable with percolating water than NH_4^+ because NH_4^+ is electrostatically attracted by negatively charged clay surfaces and functional groups of soil organic matter [24]. In contrast, NO_3^- is susceptible to leaching from the root zone [25]. Furthermore, the prevention of nitrification by plants may also have developed a greater capacity for the absorption of NH_4^+ than for NO_3^-, and the tendency of a species to take up either NO_3^- or NH_4^+ could impact the recycling efficiency and productivity of a given ecosystem [23].

The effective regulation of nitrification rates could play a key role in the dynamics of plant communities as the stimulation and inhibition of this process have been shown to strongly affect plant community structures [26,27]. Changes in the proportion of NH_4^+ and NO_3^- could influence plant communities because different plants show different preferences for the two plant-available forms of nitrogen [28].

The introduction of agroforestry tree species into croplands is considered an eco-friendly means of soil fertility enhancement for smallholder farmers. But alongside this, there is a need to understand the changes in the soil NH_4^+/NO_3^- ratio and their relationships with below and above-ground biotic and abiotic parameters under the influence of nitrogen-fixing tree species such as *Faidherbia. albida* so as to achieve sustainable soil fertility management and address environmental issues.

Faidherbia albida, hereafter *F. albida*, is an important agroforestry species introduced that is deliberately left to grow in croplands, mainly in the arid and semi-arid areas of Africa and the Middle East [29]. The tree fixes atmospheric nitrogen through symbiotic association with *Rhizobium* bacteria [30] (Teixeira, 2016), particularly with *Brady rhizobium* species [31].

Several research reports revealed the ability of *F. albida* to alleviate soil fertility problems of marginal and inherently nutrient-poor soil in the arid and semi-arid areas of the tropics [32]. A meta-analysis by Sileshi [33] provided evidence for significant increases in soil organic carbon (46%), total nitrogen (50%), phosphorus (21%), potassium (32%), and yields of maize (150%), and sorghum (73%) grown under tree canopies compared to outside canopies in inherently nutrient-poor soil.

Most importantly, *F. albida* sheds leaves during the main rainy season, but bears leaves and flowers near the offset of the rainy season, a phenomenon referred to as a reversed leaf phenology, and this unique feature makes the tree ideal for agroecosystems. In addition to enhancing soil fertility and increasing crop yield, the tree is also used as a source of firewood and fodder production by rural communities [34,35].

Though there are dozens of research reports related to the effect of *F. albida* on some soil physicochemical properties, growth, and the yield of some field crops [33], none of them address the issue of NH_4^+/NO_3^- ratios under varying stand ages of *F. albida*.

The study area of the Abraha-Atsbeha village in Tigray, northern Ethiopia, became globally recognized, and the community obtained an award internationally for the restoration of formerly degraded lands through continued soil and water conservation and afforestation works [36]. As a result, vegetation cover, groundwater, and irrigable portions of arable lands increased [37].

Considering the agricultural, ecological, and environmental importance of nitrogen forms, the present study aimed to examine changes in NH_4^+/NO_3^- ratios and correlate these changes with some selected soil bio-physicochemical properties in response to the influence of different *F. albida* tree age classes. Our research report addressed the hypothesis that the soil NH_4^+/NO_3^- ratio could change, as influenced by varying ages of *F. albida* trees.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in the Mendae micro watershed of Abraha-Atsbeha village in Tigray, Ethiopia, under a scattered *F. albida* agroforestry. The watershed is geographically located between 39°30′ E and 39°45′ E longitude and 13°45′ N and 14°00′ N latitude with an elevation range of 1900 m–2600 m above sea level (Figure 1).

The Abreha-Atsbeha watershed is divided into Mendae, Weyni, and Arato micro watersheds with a total area of 6766 ha. These micro watersheds are channeled to the Suluh River, which is the longest and the only perennial river in the study area [38]. Of the mentioned micro watersheds, the Mendae micro watershed was chosen to be the specific study site. The selection criteria for this watershed were as follows: (1) homogenous parent material (Adigrat sandstone) from which the *Arenosols* were formed, (2) uniform and gentle slope area, (3) and the abundance and availability of different stand ages of *F. albida* trees.

The landscape of the study area is mostly hillside and mountainous (45.5%) with a 21.5% medium slope, and the rest have a 34% gentle slope used for crop cultivation [39]. Abreha-Atsbeha has a semi-arid agroecology with an average temperature of 17–23 °C and an average annual rainfall of 375–788 mm (Figure 2).

The natural vegetation at the adjacent mountains of Abraha-Atsbeha is composed of *Vachellia etbaica*, *Euclea racemosa*, *Rhus natalensis*, *Carissa spinarum*, and *Maytenus senegalensis*, but *F. albida*, *Vachellia seyal*, and *Ficus vasta* are the dominant tree species at the valley bottoms.

The predominant soil type of the study area is *Arenosols* (67.25%), followed by *Fluvisols* (25%) and *Vertisols* (7.75%) [39]. The land use types in the study area are cropland (1047 ha; 15.5%), forest land and areas of ex-closure (4325 ha; 64%), grazing land (206 ha; 3%), and (1188.25 ha; 17.5%) land occupied by houses, roads, and waterways.

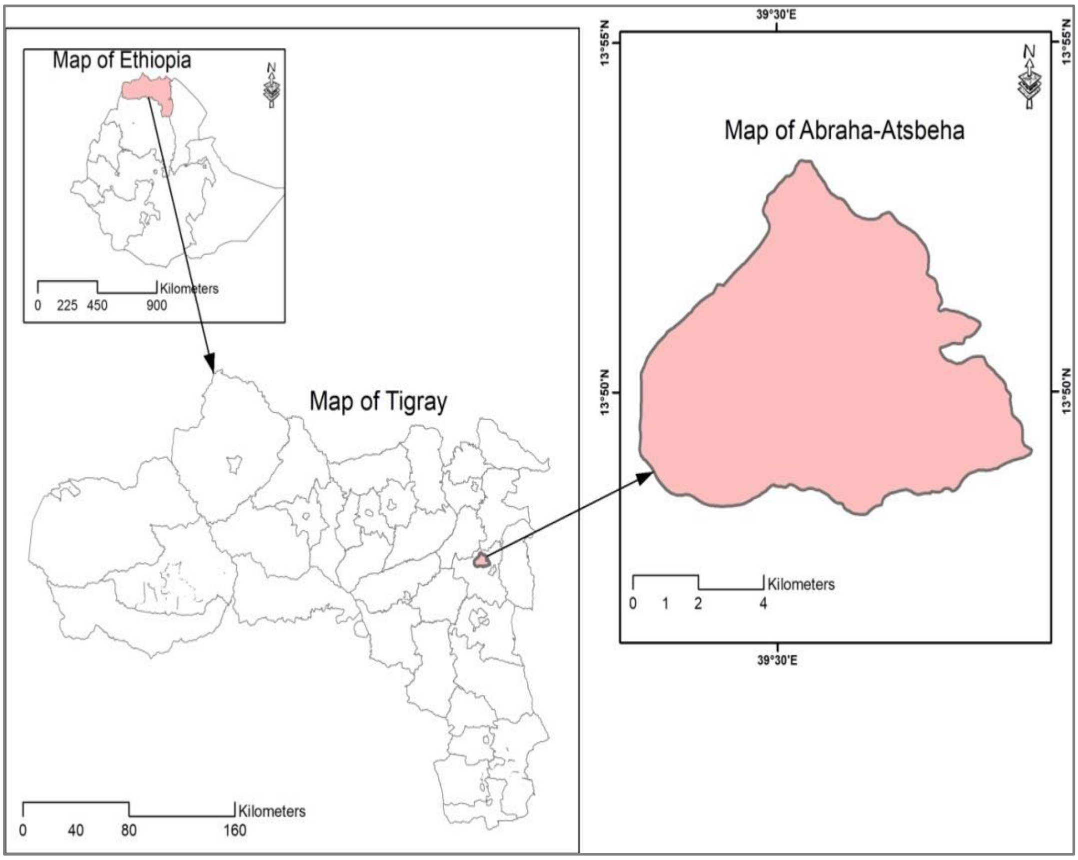


Figure 1. Location map of the study area showing the study village.

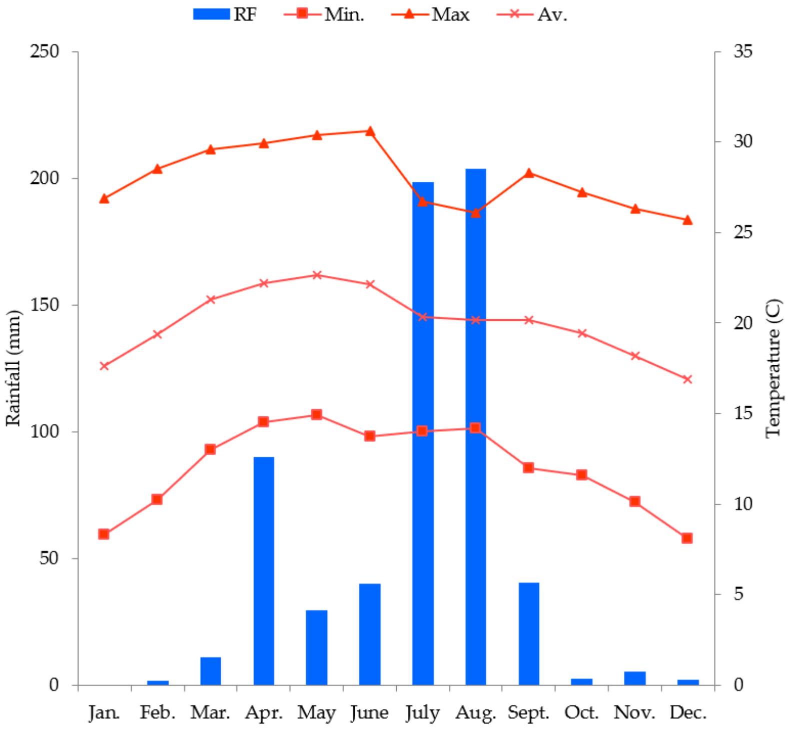


Figure 2. Climatic conditions of the study area in which bars stand for rainfall in mm and the lines stand for minimum, average, and maximum temperatures in degrees celsius (C). Monthly values of rainfall and temperature are the average of seven years (2013–2019).

The villagers are smallholder farmers, and like the other rural communities of Tigray, the livelihood of the community depends on the mixed farming of crop production and animal husbandry. In the study area, there is a scattered agroforestry system of *F. albida*.

In the scattered agroforestry system, the smallholder farmers practice mixed farming. They grow cereal crops (mainly finger millet, teff, wheat, maize, sorghum, and barley), grain legumes (chickpeas, ground nut, and vetch), fruit crops (guava, orange, lemon, and mango), and vegetables (tomato, potato, hot pepper, cabbage, lettuce, and Swiss chard). In addition to crop production, they also breed livestock (cattle, goat, sheep, and donkey) and poultry and participate in honeybee production.

2.2. The *F. albida* Tree Age Classes

F. albida trees age classes were labeled as young (15–20 years), medium age (35–40 years) or old (>60 years). The age of the trees was obtained from farmland owner farmers. The trees were selected purposefully based on the following criteria: (1) the trees must be grown on nearly level (0–3%) slopes, and (2) the canopy of the selected tree must be far away from other trees' canopies by at least twice its canopy radius.

The circumference of the *F. albida* trees was measured using a plastic tape meter, and the diameter at breast height (DBH) was calculated. The age, circumference, and DBH of the trees are presented in Table 1.

Table 1. Circumference, diameter at breast height (DBH), and tree age classes.

F. albida **Tree Age Classes (Years)**	**Average of Five Trees**	
	Cirf (cm)	**DBH (cm)**
Out-of-canopy	N/A	N/A
15–20	117.6	37.446
35–40	230	73.244
>60	438	139.484

Cirf = circumference, DBH = diameter at breast height, N/A = not applicable.

2.3. Soil Sampling

Soil samples were collected from 0 to 15 cm and 15 to 30 cm soil depths following auger and core sampling techniques in October 2020 after farmers harvested their crops. Soil samples collected from 12 sampling units of the same depth under each tree canopy were composited. Finally, we obtained 40 composite soil samples (4 levels of tree age classes $^{\times}$2 levels of soil depths $^{\times}$5 replications). Each composite sample is split into two sets of samples. One set was air-dried and sieved through a 2 mm sieve for soil physicochemical analysis. The other set was kept at 4 °C in a refrigerator until analyses of soil biological parameters were performed.

2.4. Soil Analysis

Soil physicochemical analyses were conducted in the hydrogeology laboratory, while biological parameters were performed in the botany laboratory, both located at the main campus of Mekelle University. Soil moisture content was measured using 100 g of soil that was dried in an oven (Model: 78532TUTTLINGEN, Germany) at 105 °C for 12 h. The analysis of bulk density (BD) was performed according to the core method following the procedure outlined by [40]. The setermination of soil organic carbon (SOC) was conducted based on the Walkley & Black chromic acid wet oxidation method according to a procedure developed by the global soil laboratory network [41]. Soil organic carbon was oxidized by 0.167 M of a potassium dichromate ($K_2Cr_2O_7$) solution in concentrated sulfuric acid and then quantified by the colorimetric method with a spectrophotometer (model: UV-VIS Spectrophotometer, Series No.: AE1605020) at a wavelength of 600 nm [41]. The UV-VIS was double-beamed. Concentrations of the parameters were determined in terms of absorbance and specific wavelengths. Soil particle size distributions were analyzed following the sedimentation method and measured using a hydrometer [42]. Soil pH and electrical conductivity (EC) were measured in a soil–water suspension (2:5 soil–water ratio) using a pH meter (model: HI99130, Italy) and an EC meter (model: Multi 3410 SETC, Germany). After Kjeldahl digestion in a digestion bomb, total nitrogen (TN) was also determined by the colorimetric method using the above-mentioned UV-VIS spectrophotometer. Available phosphorus (P) was extracted with 0.5 M of $NaHCO_{3,}$ and colorimetric determination was performed using the spectrophotometer. Exchangeable cations and cation exchange capacity (CEC) were analyzed according to the ammonium acetate extraction method. The concentration of calcium (Ca) and magnesium (Mg) were determined using an atomic absorption spectrophotometer (model: 50B VARIAN, Germany), while potassium (K) and sodium (Na) concentrations were determined using a flame photometer (model: JANWAY, PFP7, S/N: 9299, UK). NH_4^+ N and nitrate (NO_3^-) were extracted using 2 M of the KCl analytical grade and determined by the colorimetric method using the UV-VIS spectrophotometer.

2.5. Soil Nematode Extraction and Identification

Soil nematodes were extracted from 100 g of fresh soil for 48 h using the modified Baermann funnel method, and then the suspension was collected in a test tube, as described by Cesarz et al. [43]. After extraction, nematodes were preserved in a 4% formaldehyde solution for further analysis [44]. Nematodes were counted under an inverted Olympus microscope (Model: CKX31SF).

2.6. Analyses of Microbial Biomass and Determination of Fungi/Bacterial Biomass Ratio

Soil microbial biomass carbon was estimated by the maximum initial rate of CO_2 evolution after soil enrichment with glucose, as described by Ananyeva et al. [45]. Soil samples, 10 g from each factor level with five replications, were placed in vials (60 mL) containing a glucose solution (10 mg/g of soil basis) and then subjected to incubation at 22 °C for 24 h in a multi-room incubator (model: WIM-4, Serial No: 1001356163000). To obtain the microbial biomass for fungi and bacteria separately, a selective inhibition technique was applied using antibiotics cycloheximide and streptomycin to inhibit fungi and bacteria, respectively, as described by Ananyeva et al. [45]. According to the procedure outlined by Bailey et al. [46] (2007), antibiotics at 0.02 g/L g of the soil's basis were added 1 h earlier before the addition of the glucose substrate. After 24 h of incubation, the percentage of CO_2 was measured by inserting the needle of a portable headspace gas analyzer embedded with infrared CO_2 sensors (model 325 gas analyzer, Mocon Inc., Brooklyn Park, MN, USA) into the incubating vials through a parafilm foil seal. Calculations to quantify the respiration rate and conversion of the percentage of CO_2 to microbial biomass and to determine the fungi/bacterial biomass ratio were conducted according to Equations (1), (2) and (3), respectively, as described by Bailey et al. [46].

$$CO_2\left(\mathrm{mL\ hr^{-1}g^{-1}soil}\right) = \frac{(\%CO_2\ at\ vhs) \times (tvhs)}{1\ \mathrm{hr}\ \times 100\ \mathrm{g\ soil}} \tag{1}$$

where *vhs* is the vial headspace while *tvhs* is the total vial head space.

$$mg\ biomass\ \mathrm{C}\ 100\ \mathrm{g}^{-1}\ \mathrm{soil} = 40.04 \times (CO_2\ \mathrm{hr}^{-1}\ 100\ \mathrm{g}^{-1}) + 0.37 \tag{2}$$

$$Fungi/Bacterial\ biomass\ ratio = \frac{(A - B)}{(A - C)} \tag{3}$$

where A is the respiration rate measured (μ CO_2 h^{-1} g^{-1} soil) in the absence of inhibitors; B is the respiration rate in the presence of the fungicide; and C is the respiration rate in the presence of the bactericide.

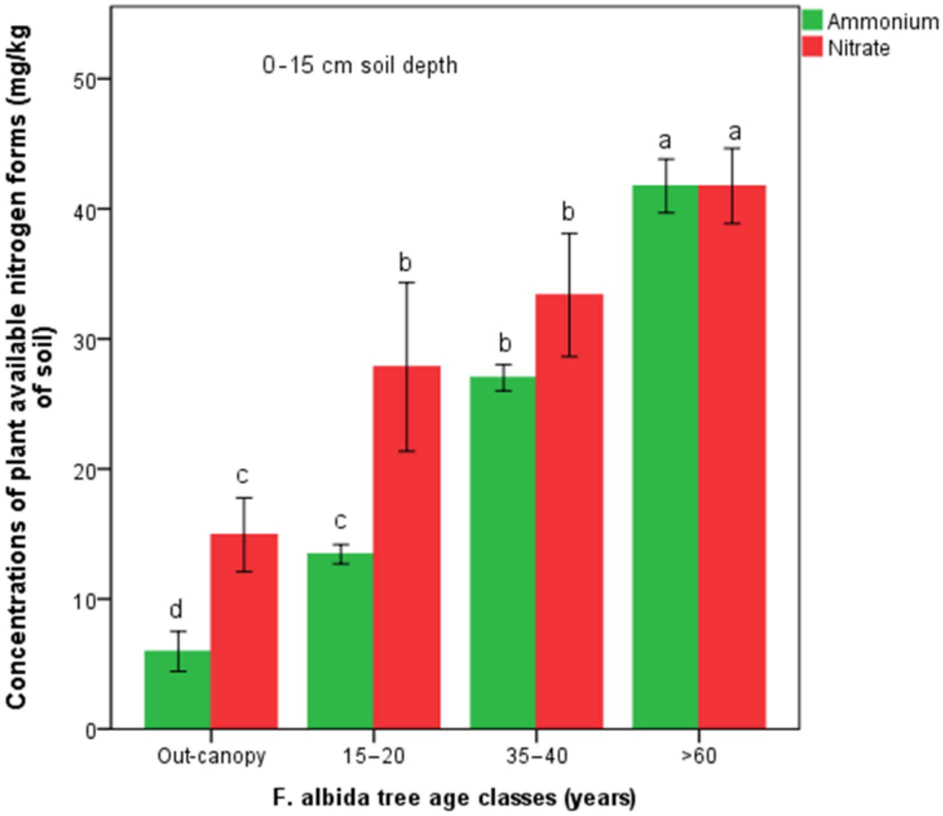
2.7. Statistical Analysis

First, normality was tested using the Shapiro–Wilk test to determine if the data sets satisfied the assumptions for parametric tests. Data sets showed normal distribution without log transformations. Hence, the studied soil physicochemical and biological parameters were subjected to parametric tests and were compared using one-way analysis of variance (ANOVA) with *F. albida* tree age classes as a grouping factor. But, since our initial analysis of factorial ANOVA showed no interaction effect between the trees' age classes and soil depths, separate analyses of one-way ANOVA were conducted for the 0–15 cm and 15–30 soil depths with the *F. albida* tree age difference factor being shared. After the ANOVA tests, post hoc tests were performed to determine the significant differences among the means using Tukey's HSD ($p < 0.05$, pair-wise multiple comparisons). Pearson correlation analyses between the NH_4^+/NO_3^- ratio and the other studied soil physicochemical and biological parameters were conducted to identify which of them was significantly correlated. All the normality, ANOVA, Post Hock, and correlation analyses were performed using SPSS statistical software version 20.

3. Results

3.1. Effects of Different F. albida Tree Age Classes on NH_4^+/NO_3^- Ratio and the Other Studied Soil Bio-Physicochemical Properties

The one-way ANOVA revealed that the concentration of NH_4^+ and NO_3^- was significantly higher in soil under medium age and old *F. albida* tree canopies than those in the out-of-canopy and the young tree age classes. For both nitrogen forms, the lowest values were quantified from out-of-canopy soil, and the highest were those influenced by the old *F. albida* tree age classes (Figure 3). Similarly, the significantly highest NH_4^+/NO_3^- ratio was measured in the old *F. albida* tree age classes and the lowest in the out-of-canopy soil (Figure 4).



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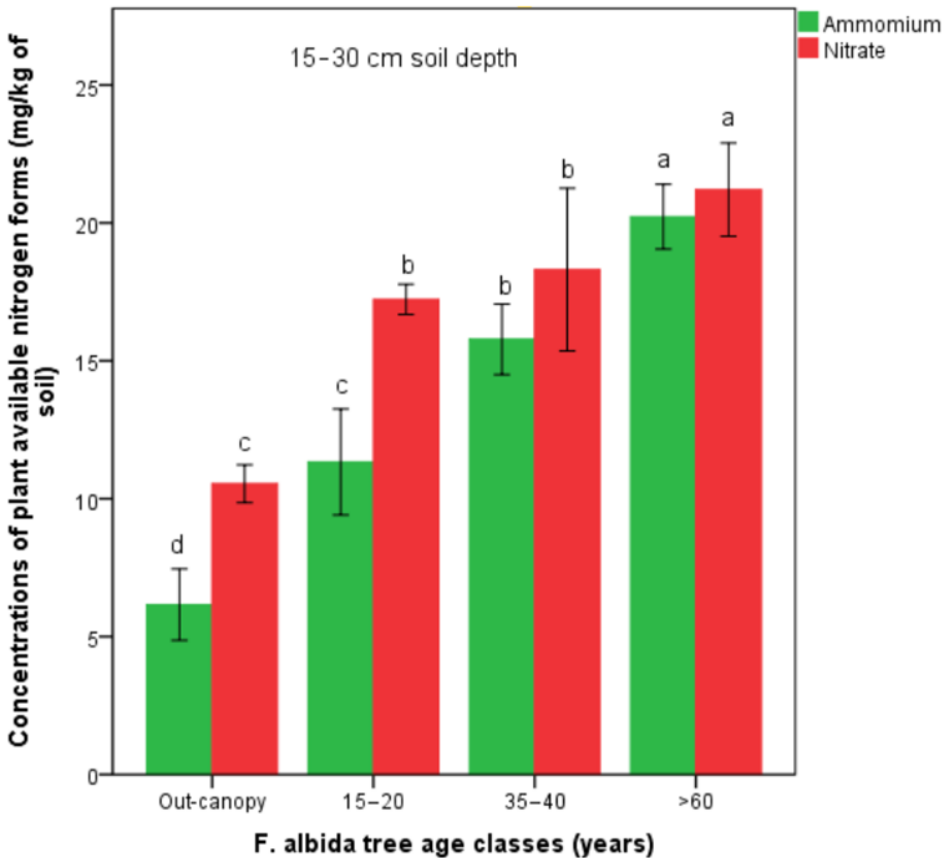
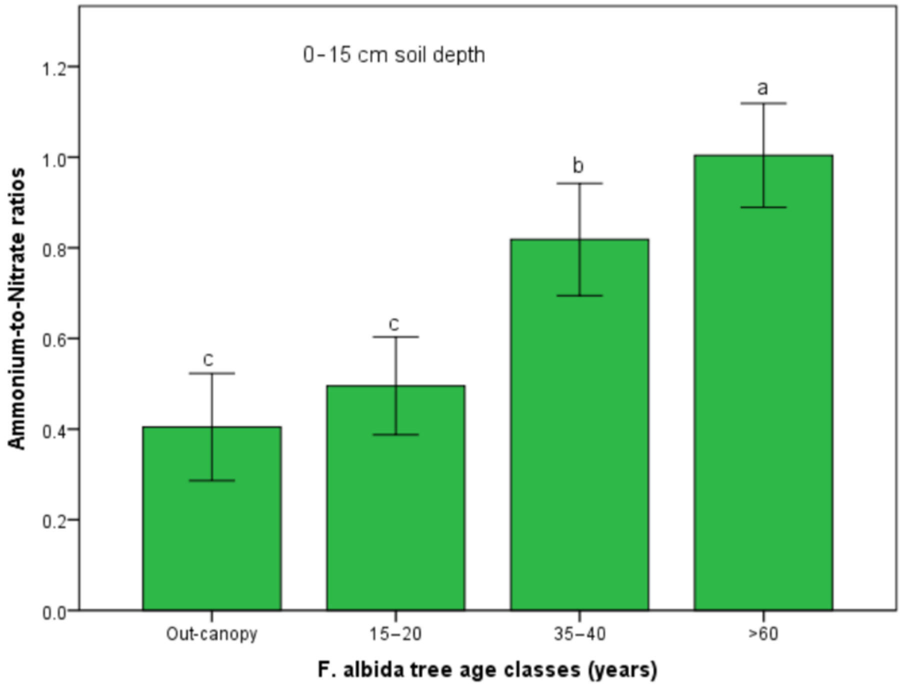


Figure 3. Changes in concentrations of NH_4^+ and NO_3^-, as influenced by different F. albida tree age classes in the 0–15 and 15–30 cm soil depths. The same nitrogen forms across the age categories are compared only with each other. Different lower-case letters indicate significant differences at $p \leq 0.05$. Values are means of 5 replications.



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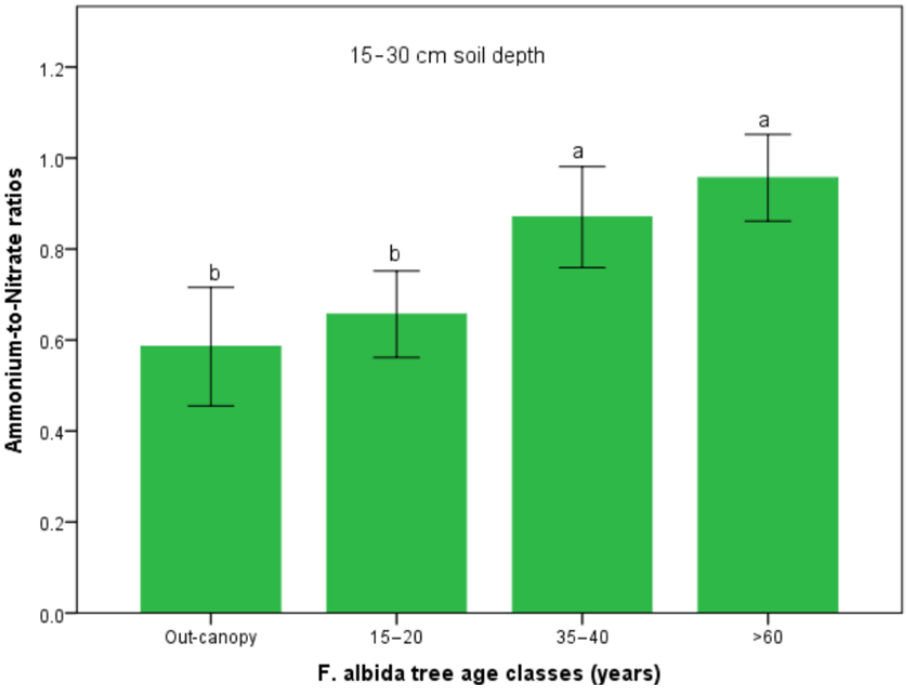

Figure 4. Changes in NH_4^+/NO_3^- ratio as influenced by different F. albida tree age classes in the 0–15 and 15–30 cm soil depths. Different lower-case letters indicate significant differences at $p \leq 0.05$. Values are means of 5 replications.

Generally, varying *F. albida* tree age classes significantly influenced the concentration of plant-available phosphorus, exchangeable bases, CEC, EC, pH, SOC, the C/N ratio, BD, moisture content, and proportion of textures (clay, silt, and sand) of *Arenosol*. Except for pH, sand content, and BD, which were shown to decline as the trees became old, all the other soil physicochemical parameters showed a significant increase as the tree age increased. In particular, significantly higher values of available P, exchangeable base cations (K, Ca, and Mg), CEC, EC, SOC, the C/N ratio, clay, and silt content were recorded in soil collected from under the canopies of the old *F. albida* tree age classes followed by the medium age and then by the young tree age classes while significantly lower values of these parameters were measured in soil collected from out-of-canopy soil (Table 2).

Table 2. Changes in soil physicochemical properties as influenced by different *F. albida* tree stand age classes for the 0–15 cm soil depth. We presented only the results for the 0–15 cm soil depth because, regardless of the magnitude of the patterns of change, both soil depths were found to be similar. (Values mean ± standard deviation connected by different lower-case letters in the same raw are significantly different at $p < 0.05$.

Variable	***F. albida* Tree Age Classes (Years)**			
	Out-of-Canopy	**15–20**	**35–40**	**>60**
TN (%)	0.2 ± 0.02 c	0.3 ± 0.02 b	0.36 ± 0.03 ab	0.4 ± 0.02 a
C/N (ratio)	4 ± 0.7 c	5.1 ± 0.9 b	5.5 ± 0.6 ab	7 ± 0.7 a
P (mg/kg)	15 ± 1.2 c	17 ± 2 c	26 ± 2 b	30 ± 3 a
Ca (mg/kg)	320 ± 57 d	550 ± 79 c	680 ± 57 b	960 ± 65 a
Mg (mg/kg)	95 ± 8 c	115 ± 8 c	162 ± 6 b	197 ± 20 a
K (mg/kg)	39 ± 1.5 d	145 ± 9 c	200 ± 10 b	481 ± 13 a
Na (mg/kg)	8.5 ± 2 d	12 ± 2 c	15.5 ± 2.5 b	20.5 ± 3 a
SOC (mg/kg)	0.8 ± 0.06 d	1.6 ± 0.08 c	2 ± 0.19 b	2.6 ± 0.5 a
CEC (meq/100g)	4.4 ± 0.4 d	7.5 c ± 0.1	10 ± 0.5 b	15 ± 1 a
pH (scale)	6.25 ± 1 a	6.2 a ± 0.08	6.15 ± 0.01 a	6 ± 0.07 b

Table 2. *Cont.*

Variable	*F. albida* Tree Age Classes (Years)			
	Out-of-Canopy	15–20	35–40	>60
EC (dS/cm)	0.3 ± 00 d	1.5 c ± 0.01	1.7 ± 0.02 b	2.3 ± 0.03 a
Sand (%)	87 ± 1 a	85 a ± 0.8	72 ± 1 b	70 ± 0.8 c
Silt (%)	7.2 b ± 1 b	7.2 b ± 0.8	13 ± 0.8 a	13 ± 0.7 a
Clay (%)	5.8 ± 1.3 c	7.2 c ± 0.8	14 ± 1.8 b	17 ± 0.3 a
BD (g/cm^3)	1.6 ± 0.04 a	1.49 a ± 0.02	1.43 ± 0.04 b	1.39 ± 0.05 a
MC (%)	0.98 ± 0.15 c	1.1 bc ± 0.3	1.3 ± 0.15 b	1.5 ± 0.11 a

TN = total nitrogen, C/N = carbon-to-nitrogen ratio, SOC = soil organic carbon, CEC = cation exchange capacity, EC = electrical conductivity, BD = bulk density, MC = moisture content.

Higher mean values for the studied parameters that increased and lower values for those shown to decrease were recorded for the 0–15 cm soil depth than for the 15–30 cm. Nevertheless, the patterns of change, as governed by the tree age class differences, were found to be quite similar for the two soil depths.

3.2. Effects of Different F. albida Tree Ages on Microbial Biomass Carbon and Abundance of Nematodes

Soil microbial biomass carbon (Figure 5), fungi/bacterial biomass (data not presented here), and abundance of nematodes (Figure 6) showed significant increases as the *F. albida* tree age increased ($p \leq 0.05$). The microbial biomass in the old *F. albida* tree age classes was found to be 5, 2, and 1.5 times higher than in the out-of-canopy, young, and medium age, respectively.

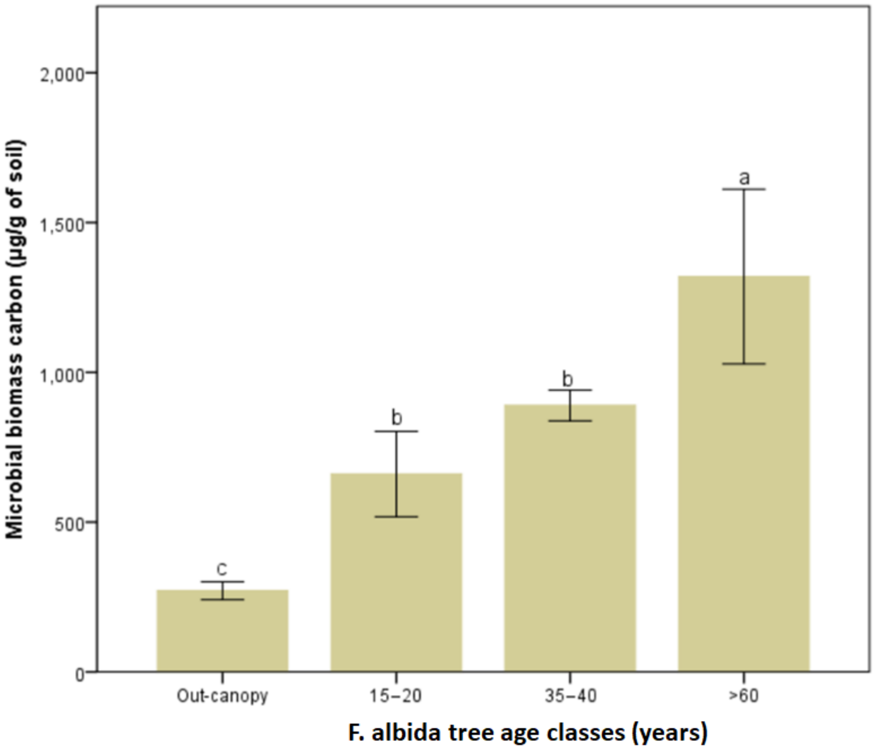

Figure 5. Changes in microbial biomass carbon (MBC) as influenced by the different *F. albida* stand age classes. Values connected by the same lower-case letters are not significantly different at $p \leq 0.05$. Values are means of 5 replications.

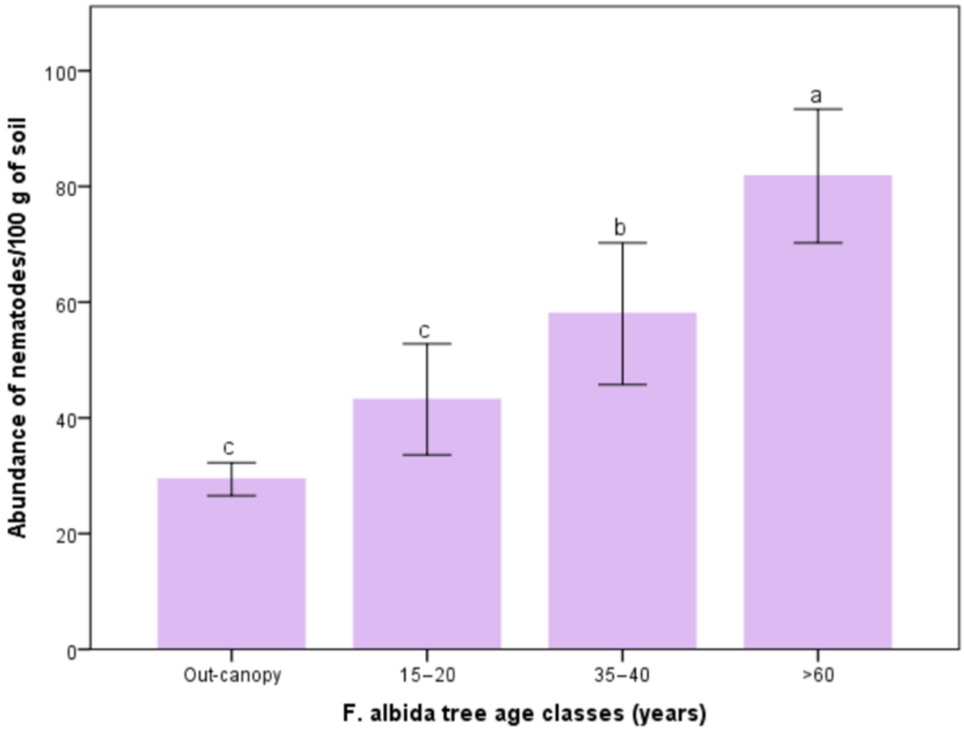

Figure 6. Changes in abundance of nematodes, as influenced by the different *F. albida* tree age classes. The mean number of nematodes connected by the same lower-case letter is not significantly different at $p \leq 0.05$. Values are means of 5 replications.

3.3. Correlations between NH_4^+/NO_3^- Ratio and Soil Bio-Physicochemical Properties

The NH_4^+/NO_3^- ratio showed strong positive correlations with the percentages of clay and silt, abundance of nematodes (AN), fungi/bacterial biomass ratio (F/B), CEC, microbial biomass carbon, TN, and soil organic carbon. In contrast, it showed strong negative correlations with the percentage of sand, BD, and pH (Table 3). Generally, the NH_4^+/NO_3^- ratio showed positive correlations with parameters that became higher as the *F. albida* trees became older and negative correlations with lower parameters.

Table 3. Pearson correlations between NH_4^+/NO_3^- ratio and some selected soil bio-physicochemical properties.

	Clay	Silt	AN	F/B	CEC	MBC	TN	SOC	Sand	BD	pH
NH_4^+/NO_3^-	0.91 **	0.91 **	0.90 **	0.90 **	0.88 **	0.86 **	0.83 **	0.83 **	−0.94 **	−0.81 **	−0.74 **

Where: ** = Correlation was significant at 0.01 alpha levels.

4. Discussion

The results support the hypothesis that the *Arenosol* NH_4^+/NO_3^- ratio can change, as influenced by varying *F. albida* tree age classes. The tree age difference also affected all the studied soil physicochemical and biological parameters. As a result, varying *F. albida* tree age classes brought significant changes in the NH_4^+ and NO_3^- balances. The findings indicated that both the plant-available nitrogen forms became higher and higher as trees became older and older. *F. albida* is among the nitrogen-fixing trees through its symbiotic association with *Rhizobium* bacterial species [47]. Hence, the increment in the nitrogen forms in soil from under the canopies of *F. albida* might be due to the addition of biologically fixed nitrogen by the tree and its symbiotic microbial partners. According to Hazelton & Murphay [48]'s ratings for plant-available nitrogen in soil and crops and their response to nitrogen fertilizer, our soil test results for the plant-available forms of nitrogen in soil under *F. albida* tree canopies are in line with moderate-to-high-level ratings.

The significant increase in the NH_4^+/NO_3^- ratio in soil under old *F. albida* tree canopies may suggest that the soil conditions under old trees are in favor of ammonification rather than nitrification. Indirectly, this result implies that different *F. albida* tree age classes affect the activities of ammonifying and nitrifying bacteria differently. Some agroforestry tree species have been reported to inhibit soil nitrification by releasing gallocatechin and catechins during the decomposition of leaf litter [49]. Slow rates of nitrification in forest ecosystems have been considered to be an indicator of ecosystem maturity [50]. Our results have shown that NO_3^- is the dominant plant-available form of nitrogen in out-of-canopy soil, but the ratio of NH_4^+/NO_3^- showed a significant increase in soil under the young and medium-age tree canopies of *F. albida* and eventually approached one in the old trees. The increase in the NH_4^+/NO_3^- ratio may also be due to the increased NH_4^+ holding capacity of the soil under the tree canopies. NH_4^+ can be adsorbed by organo–mineral complexes and, hence, retained in the soil profile. In contrast, the negative charge in NO_3^- makes it susceptible to leaching [51].

Our results have shown strong and positive correlations of the NH_4^+/NO_3^- ratio with clay content, F/B ratio, CEC, and SOC but negative correlations with pH, the percentage of sand, and BD. The higher clay content, CEC, and SOC in soil under old *F. albida* trees may contribute to retaining NH_4^+ in soil particles. The higher F/B ratios in the soil under old *F. albida* trees indicate the increment of fungi communities, which are known to inhibit nitrifying bacteria with their extracellular secretions [52] (Dudáš et al., 2022). The slight decrease in soil pH under old *F. albida* trees may also contribute to the increase in the NH_4^+/NO_3^- ratio because a lower pH is known to inhibit the process of nitrification [53].

Therefore, the increased NH_4^+/NO_3^- ratio under tree canopy soil with increasing *F. albida* tree age might be due to the inhibition of nitrification by the relatively lower pH under the canopies of old *F. albida* trees.

The slight lowering of the soil pH under old *F. albida* trees might be due to greater additions of organic acids from the increased organic matter derived from the tree and microbial biomass that was shown to increase as the tree age increased. An earlier study by Sahrawat et al. [54] revealed that the rate of nitrification decreased as soil pH became lower, and they found that nitrification did not take place in forest soil under natural pH (5.0). Soil pH is the major factor regulating the nitrification process in soil [55]. Nitrification takes place in the soil at a pH ranging between 5.5 and 10.0, with the optimum approximately around 8.5 [55,56].

According to Hazelton & Murphy [48] ratings for TN, we analyzed medium and high levels of TN in soil under the influence of medium age and old *F. albida* tree age classes, respectively. The TN results of the medium age and old *F. albida* tree age classes were in agreement with Gebirehiwot et al. [57] for soil influenced by *F. albida*.

Organic matter is the most important fraction of soil, which directly and indirectly influences the biological, chemical, and physical properties of soil [58,59]. Proper functioning and ecosystem services of soil largely depend on their organic matter content [60,61]. Thus, in our study case, the tremendous changes in the biological and physicochemical variables along *F. albida* tree age gradients might be primarily due to the additions of quality (in terms of the carbon-to-nitrogen ratio) organic matter derived from the tree. As a result, the MBC, F/B ratio, SOC, AN, and C/N ratios significantly increased as the tree age classes increased.

Compared to Hazelten & Murphay's ratings for available P [48], our soil test results for available P indicate low values in out-of-canopy soil, moderate values under young and medium-age trees, and high values under old *F. albida* tree age classes. Like the nitrogen forms and plant-available P, the concentration of readily available base cations, CEC, and EC all increased as the tree age increased. The increase in exchangeable base cations, CEC, and EC could be attributed to the increase in soil organic matter as the tree became older because organic matter contributes more to retaining cationic nutrients. Although it is well understood that soil texture and bulk density are among the relatively static variables [62], our results showed a significant increase in clay and silt percentages in the medium-age and old *F. albida* tree age classes. The changes in the percentage of clay and silt may reflect the role of *F. albida* trees in facilitating pedogenetic processes, such as transformations of primary to secondary clay minerals and their retention in the soil profile.

Our results also indicate that the varying *F. albida* tree age classes influenced soil moisture content and bulk density differently. Soil moisture content showed a pattern of increase with the tree stand age, while bulk density was shown to decrease. The decreased soil bulk density and increased moisture content may be due to the increased soil organic matter in the under canopy of *F. albida* trees, whereas the increased moisture content may be due to the increased water-holding capacity of soil because of the increased soil organic matter as the tree becomes older.

5. Conclusions

This study confirms that the soil NH_4^+/NO_3^- ratio becomes significantly higher as the *F. albida* tree age increases. The NH_4^+/NO_3^- ratio showed strong positive correlations with percentages of clay and silt mineral fractions, AN, F/B, MBC, CEC, TN, and SOC, but strong negative correlations with percentages of sand, BD, and pH. Indirectly, the results indicate that the soil conditions under *F. albida* trees are in favor of ammonification rather than nitrification, and the degree of mineralization is higher than NH_4^+ oxidation by nitrifying bacteria.

Generally, the increment in the soil NH_4^+/NO_3^- ratio under canopies of old *F. albida* trees suggests that these trees are not only contributing to sustainable soil fertility management but also reducing environmental pollution.

Finally, the authors recommend further investigations, especially regarding changes in the community structure of ammonifying and nitrifying bacteria in response to different ages of *F. albida* trees and other agroforestry plant species.

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