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Effects of artificial nitrogen deposition on the forest floor and soil chemistry under chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey) plantation in the north of Iran

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Highlights

1. Our estimate of ambient N fluxes was 27 and 17 kg ha^{-1} $year^{-1}$ in the form of NO_3^--N and NH_4^+-N, respectively.
2. The nutrient concentrations in the forest floor (N, P) were increased in the N treatment.
3. N deposition decreases soil pH, available K, available P, and microbial biomass C.
4. N deposition increases soil EC, OC, N, and enzyme activity.
5. Our results were obtained with greater simulated deposition rates by extra N input compared with ambient rates, but annual ambient N deposition fluxes was high in our study area. It is thus realistic to expect that ambient N deposition causes similar changes within a few years.

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Abstract:

Human demand for food and energy has led to significant changes in the level of reactive nitrogen (Nr) released to the atmosphere and then deposited in the biosphere. This study aimed to investigate artificial N deposition's impact on the forest floor and soil chemical properties in an oak (*Quercus castaneifolia* C. A. Mey) plantation in the north of Iran. Twelve plots of 200 m^2 (20 m × 10 m) were set up in the study area. Four N treatments were considered: zero (control), 50 (low), 100 (medium), and 150 (high) kg N ha^{-1} $year^{-1}$. N in the form of NH4NO3 solution was manually sprayed onto the understory plots monthly for one year. The total N, P, K, and organic C (OC) of the forest floor were measured. Soil N, available P, available K, pH, EC (Electrical Conductivity), OC, microbial biomass C (MBC), and urease enzme activity were measured in the 0-10 cm depth. The concentration of total N and P of the forest floor was significantly higher in the high N treatment. The total concentration of N (+36%), the urease activity (+44%), and EC (+12%) of soil increased with raising the high-N treatment compared to the control, but the MBC (-20%), available P (-28%), and available K (-15%) were significantly reduced in the high-N nitrogen treatment. Our results were obtained with simulated deposition rates that exceed ambient fluxes, but ambient N deposition is nevertheless high in our study area.

Keywords: ammonium nitrate, soil chemistry, microbial biomass carbon, urease enzyme activity, forest floor

Introduction

The Hyrcanian forests are located along the southern coast of the Caspian Sea. With an area of 1.8 million hectares, these forests consist of 50 shrub and 80 trees species. Besides wood production, the forest provides many essential ecosystem services, including climate and water regulation, as well as opportunities for eco-tourism (Sagheb-Talebi et al. 2004). The forest area in the north of Iran has decreased from 3.4 million hectares in 1946 (Marvi mohajer 2005) to 2.1 million hectares in 2020 (Anonymous 2020). Land-use change is a leading cause of the decline of the Hyrcanian forests areas; the common types of land-use change in northern Iran are the transformation of forest into agricultural lands (Asadiyan et al. 2013), and arable lands have increased by about 37% in this region. N fertilization and animal husbandry in the converted area are a source of N deposition in the Hyrcanian region, which can change its N cycle. Like in other regions of the world, with the expansion of fertilizer use and fossil fuel combustion, more reactive nitrogen (N) enters the terrestrial ecosystems via atmospheric N deposition (Galloway et al. 2008). Compared with biologically fixed N produced, atmospheric N deposition is becoming a more significant source of N for terrestrial and aquatic ecosystems (Galloway et al. 1995).

Increased inputs of N severely impact terrestrial ecosystems, especially in forests. The global N deposition rate is expected to reach 200 Tg/yr by 2050 (Galloway et al. 2008), which is twice the current level. Nitrogen deposition affects forests in many aspects, including tree health, soil productivity and C sequestration (Levy-Booth et al. 2014; De Vries et al. 2014), soil exchangeable cations and biodiversity (Bobbink et al. 1998; Shi et al. 2018) and water quality (Schleppi et al. 2017). Recent studies indicate that N deposition also affects some ecological properties and processes of the forest such as soil microbial biomass and enzyme activities (Zhou et al. 2018; Li et al. 2021), respiration (Zhang et al. 2019; Sun et al. 2019; Forsmark et al. 2020, Tafazoli et al. 2021), pH and nutrients (Lu et al. 2014; Mao et al. 2017; Tafazoli et al. 2019). In recent decades, the impact of high atmospheric N deposition has been studied in many cases in Europe and North America (Pardo et al. 2011; Erisman et al. 2015; Forsmark et al. 2020), and East Asia (Wang et al. 2017). Asia is now the global hotspot of N deposition. Since the early 2000s, the highest rates of global N deposition have been documented in various regions of Asia, including China, India (Gao et al. 2020; Zhang et al. 2021), Japan, and South Korea (Hu et al. 2019). Some parts of Indonesia, China, Vietnam, Thailand, and Malaysia are hotspots of wet N deposition (24–102 kg N ha^{-1} yr^{-1}) in Asia (Zhang et al. 2021). Nevertheless, observational studies on spatial distributions of N deposition for the entire continent are scarce (Duan et al. 2016), especially in Iran, where little is known about N deposition and its effects. There is only one study about the effect of N deposition on soil base cations and net N mineralization (Tafazoli et al. 2019), and there is no report about the effects of N deposition on the forest floor and soil chemistry. Due to the global significance of N deposition, the scarcity of comparable data on N deposition and its effects in Iranian forests, and considering the costs of measures for reducing N emissions, we need more data and more extended monitoring of N ambient deposition and its impact.

The present study aimed to identify the changes in soil properties under artificial N deposition in an oak plantation. Chestnut-leaved oak (*Quercus castaneifolia*) as a high-quality tree accounting for 7.4% of the total number (fifth species) and 7.8% of the volume (fourth species) in Hyrcanian forests (Gorji Bahri et al. 2013). Chestnut-leaved oak is a popular and common tree species for reforestation in this region. The specific objectives of our research were: (1) to estimate the ambient atmospheric deposition by measuring throughfall chemistry and the corresponding nutrient fluxes, especially for inorganic N (NO_3^--N and NH_4^+-N), (2) to measure the forest floor and its nutrient contents, and (3) to examine which factors of soil chemical characteristics is most affected by the nitrogen addition experiment. We hypothesized that the N addition experiment would alter chemical features of the forest floor and that N deposition would increase acidity and changes in chemical characteristics (urease and MBC), even in the short term.

Materials and method

Study area

This study was conducted in a *Q. castaneifolia* plantation located in the central part of the Hyrcanian forests, northern Iran (latitude: 36°29′20″ to 36°29′38″ N, longitude: 53°0′40″ to 53°1′14″ E, and elevation: 400 m above sea level). Fifteen km away from this plantation, there is a meteorological station with several years of history; in this study, we used its 29-year data. The mean annual temperature and precipitation are 17 °C and 950 mm, respectively, with the highest amount of rainfall in February (wet season) and the lowest amount in July and September (dry season). The original forest was a mixed temperate stand consisting of several native hardwood species such as chestnut-leaved oak (*Q. castaneifolia* C. A. M.), ironwood (*Parrotia persica* C. A. M.), and hornbeam (*Carpinus betulus* L.), which was degraded during the last decades. In 1986, this area was clear-cut and then reforested. The herbaceous species covered less than 10% in the plots and included butcher's broom (*Ruscus hyrcanus* Woronow), perforate St John's wort (*Hypericum perforatum* L.), sweet woodruff (*Asperula odorata* L.), and ann ala *(Hedera pastuchovii* Woronow). The oak monoculture considered for the present investigation was located in a relatively flat area (≤ 5 % slope). According to the USDA soil classification, the soil type is Alfisols (derived from calcareous and dolomite rock). The plantation was 34 years old, and the planting distance was 3 × 3 m. The average diameter and height of the trees were 30 cm and 20 m, respectively. This plantation was located about 15 km northwest of the administrative center of Mazandaran Wood and Paper Company, and approximately 6 km southwest of Sari, in Mazandaran Province. From the north, it was limited by the agricultural land (paddy fields), from the south to the Perchink series forests, from the east to the Mahdasht series forests, and from the west to the agricultural lands (Anonymous 2011) (Fig. 1).

Nitrogen addition experiment

The N addition experiment design is described by (Mo et al. 2008; Tian et al. 2019). Twelve rectangular sample plots of 20 × 10 m were established as a randomized block design in the plantation. A distance of 10 m between the plots was kept as a buffer (Ma et al. 2018). According to the results of Salehi et al. (2014), wet deposition is about 56 kg N ha^{-1} $year^{-1}$ in Hyrcanian forests for elevations of 300 m. Four levels of ammonium nitrate NH_4NO_3 additions were chosen as treatments and applied in three replicates: of zero (Control, C), 50 (Low, L), 100 (Medium, M), and 150 (High, H) kg N $ha^{-}1$ yr^{-1}. NH_4NO_3 was dissolved in 20 L of water, and the solution was monthly sprayed below the canopy (understory) of each plot during one year from October, 2017, to September, 2018; in the control plots, 20 L of water was sprayed.

Throughfall

For throughfall collection, three plastic collectors (9 cm in diameter and 25 cm in height) were installed at fixed positions 1 m above the forest floor in each plot. Throughfall was measured and sampled after each rainfall event and the cumulative throughfall amount was accounted in each sample point per month for one year during the study period. For chemical analysis, the samples were combined for each season, i.e., over three months, from autumn 2017 (October-December) to summer 2018 (July–September). After collecting throughfall samples, the collectors were cleaned with distilled water before being replaced. The samples were then transferred to the laboratory and stored at 4° C. In the laboratory, the pH and EC of the throughfall water samples were measured with pH and EC meters. The nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were measured using the copper–cadmium reduction (Skalar Method) and Berthelot reaction methods (Skalar Method), respctively (Hojjati et al. 2009). A spectrophotometer was used to measure phosphate (PO_4^{3-}). The concentrations of potassium (K^+) were determined using the flame photometer (Jafarihaghighi et al. 2003). We calculated quarterly fluxes of NO_3^-, NH_4^+ in the throughfall by multiplying the concentrations by the precipitation volume (Hojjati et al. 2009).

The forest floor and soil sampling

Three samples of forest floor and upper mineral soil (0-10 cm) were taken seasonally per plot in autumn (November 2017) and winter (February 2017) (dormant seasons), as well as spring (May 2018) and summer (August 2018) (growing seasons) using a soil corer (8 cm in diameter).

In the laboratory, the water content of the soil samples was determined via drying, and soil texture was measured by the hydrometer method. Chemical properties of the soil, including pH and EC, were measured in a suspension (soil-to-water ratio of 1:2.5). Soil organic C, total N, available P, and available K were measured using the Walkley and Black procedure (Allison 1975), Kjeldahl, Olsen extraction (Olsen et al. 1954), and ammonium acetate extraction (Bower et al. 1952), respectively. Urease activity (EC 3.5.1.5) was measured after an incubation of 2 h at 37°C using a 200 mM urea as a substrate. Microbial biomass carbon (MBC) was measured by the fumigation-extraction method (Brookes et al. 1985; Sparling et al. 1990). The forest floor samples were ground with a mill, passed through a sieve of 0.5 millimeters, and analyzed for the main chemical elements. P was measured by a spectrophotometer, K using a flame-photometer, total N by the Kjeldahl method (Bremmer et al. 1982), and C by the furnace (combustion) method (Nelson and Sommers 1982).

Statistical analysis

The Shapiro-Wilk test was run to check the normality of the variables, and Levene's test was performed to examine the homogeneity of the variances. Repeated measures analysis of variance (ANOVA) was used to determine the effects of N treatments (C, L, M and H), sampling time (autumn, winter, spring and summer) and their interactions on the throughfall, forest floor and soil properties. Repeated-measures pairwise comparisons with Bonferroni adjustment was performed to determine the effect of seasons on the throughfall, forest floor and soil properties. One-way ANOVA with LSD test was applied for the effects of N treatments on the forest floor and soil properties in every season. All analyzes were performed in SPSS 22.0 statistical software. Principal component analysis (PCA) was used to analyze the relationships between the forest floor and soil properties using PC-ORD 5.0. Finally, Pearson correlation analyses were performed to examine the relationship between soil and the forest floor properties with PCA components.

Results:

Throughfall

During our study period, the highest throughfall amount was in winter, and the lowest amount was in summer. The amount of throughfall in winter (average of 3 months; 96 mm) was significantly higher than in the summer (average of 3 months; 19 mm) (see supplementary material S1). The pH of throughfall was significantly (P <0.01) higher in the summer and spring (growing season) than in the winter and autumn (dormant season) (see supplementary material S2; Fig S2.1). EC was significantly higher in the spring and lower in the winter (see supplementary material S2; Fig S2.2). The concentrations of NH_4^+, PO_4^{3-} and K^+ in throughfall were significantly higher in the growing season (spring and summer) than in the dormant season (see supplementary material S2; Fig S2.3, S2.4 and S2.5). The lowest amount of these ions was in the winter. In the summer, the concentration of NO_3^- was significantly higher in throughfall (Table 1; see supplementary material S2; Fig S2.6).

The annual throughfall amount was similar across the four N treatments (see supplementary material S3; Fig S3.1and S3.2). Throughfall fluxes all nutrients, were highest in winter. The estimated N fluxes in the form of NO_3^- were 27 kg ha^{-1} $year^{-1}$ and in the form of NH_4^+ 17 kg ha^{-1} $year^{-1}$, respectively. Inorganic N deposition rates (NH_4^+-N, NO_3^--N) fluctuated sharply over the year, with a peak occurring in winter.

Nutrient concentrations in the forest floor

In autumn and winter, the N concentration in the forest floor was statistically different across the N treatments compared with the C plots. Later, in the summer, they increased in the high-N treatment compared to the control (Fig. 2a). P concentrations in the control were highest in spring and summer, and the lowest value was measured in winter. N treatments had a significant (P <0.01) effect on the concentration of P in the forest floor. The highest concentration of P was observed in the high-N treatment in summer. N treatments had no significant (P >0.01) effect on P concentration in autumn and winter, but had a significant effect (P >0.01) in the spring (H > M > L> C) and summer (H > M > L> C) (Fig. 2b). As for the concentration of K in the forest floor, nitrogen treatments had no significant effect (P >0.01) in the autumn. However, in spring (H > C > M < L) and summer (L > H > M > C), the K concentration was significantly greater in low-N treatments than in other treatments (Fig. 2c). As for the organic C in the forest floor, nitrogen treatments had no significant effect (P >0.01) in the autumn and summer, but in the winter and spring, carbon was significantly higher in the medium- and high- N treatments than in the control (Table 2; Fig. 2d).

Soil properties

Soil physical and chemical properties were measured at the beginning of the period and before applying N treatments in the Q. *castaneifolia* plantation. The results showed that the soil texture was sandy-loamy. The soil moisture content at sampling time was 29.51%, bulk density: 1.35 g cm^{-3}, pH: 6.1 (1:2.5 H_2O), EC: 0.49 dS/m, and OC content: 4.8 % (Table 3).

The N treatment significantly decreased soil pH. The highest pH values were recorded in winter and autumn in the control. The lowest values belonged to the medium-N and high-N treatments in spring and summer

(Fig. 3a). The N treatment significantly increased the soil EC (H > M > L > C). The lowest EC was observed in control plots in winter and autumn (Fig. 3b). N treatments significantly increased the concentration of N in the soil (H > M > L > C) (Fig. 3c). The maximum total N concentrations were observed in summer and spring. The concentrations of available P and available K had a decreasing cycle over the four treatments from autumn 2017 to summer 2018 (C > L > M > H). The minimum concentration of available P was observed in H and M treatments in summer and spring, respectively. The highest available P concentration was observed in the C treatments in summer and spring. The concentration of available K in nitrogen treatments was significantly ($P < 0.01$) decreased in the N-treated plots in all seasons, but the lowest available K concentration was observed in H and M treatments in spring (C >L > M > H) and summer (C > L> M > H) (Fig. 3d and e). In the control plots, the maximum amount of carbon was observed in summer and spring. Nitrogen treatment significantly affected the soil C content in all four seasons. Especially in the summer, soil organic C was significantly elevated in the high-N application (Table 4; Fig. 3f).

The soil microbial C showed a clear trend over the four N treatments, with a minimum in the winter and a maximum in summer. While microbial C was similar across the N treatments in the autumn and winter, it significantly decreased with the rate of N application in the spring and summer (Table 4; Fig. 4a). The urease activity was higher in autumn than other seasons, and N treatments had a significant positive effect on it throughout the year (Table 4; Fig. 4b).

The results of the PCA revealed that the first and second axes represented 50.1% and 10.8% of the total variance, respectively. The right PC1 belongs to soil N, urease activity, the forest floor N, P, and soil organic C. The left PC1 indicates a condition with the accumulation of MBC, soil available P, soil pH, C/N ratio, and soil available K, and this can be attributed to the decreasing trend of these properties when increasing the nitrogen saturation in the oak plantation (see supplementary material Table S1; Fig. 5). The maximum and minimum relative changes were observed in high- and low-N treatments, respectively (Fig. 6).

Discussion

N addition and throughfall chemistry

In the present study, throughfall pH values were moderately acidic during the growing season. This demonstrates the ability of the tree canopy to reduce the acidity of precipitation, which is consistent with the results of Devlaeminck et al. (2005) and Salehi et al. (2016). During the growing season, ion exchange processes in the leaf tissue accounted for the uptake of protons and leaching of base-cations (Roelofs et al. 1989), which reduces the acidity of throughfall. The potassium content is particularly susceptible to canopy exchange, especially during the growing season (Van Ek and Draaijers 1994; Hojjati et al. 2009). In addition, our results about NO_3^- and NH_4^+ concentrations in throughfall showed that the highest NO_3^- and NH_4^+ concentrations were measured in the growing season (Houle et al. 1999) due to the wash-off of dry-deposited compounds on foliage (Salehi et al. 2016).

All the nutrients, including inorganic N (NH_4^+, NO_3^-), displayed a seasonality with the highest fluxes in the winter. Similar to previous reports (Zhang et al. 2008; Li et al. 2015), this can be ascribed to the volume of the throughfall itself. In our study, the estimated N fluxes were 27 and 17 kg ha^{-1} year^{-1} for NO_3^--N and NH_4^+-N, respectively (44 kg N ha^{-1} year^{-1}). Salehi et al. (2014) reported that about 60 kg N per hectare was annually deposited in the Hyrcanian region. This means that our measurements are in line with the values found in the same region and can be compared to the high deposition rates measured in Indonesia, China, Vietnam, Thailand, Japan, and Malaysia. As a comparison, in Europe wet N deposition ranges between 0.5 and approximately 20 kg ha^{-1} year^{-1} (Thimonier et al. 2019, Zhang et al. 2021). The primary source of NH_4^+ is agriculture, and the main source of NO_3^- is the emission of NOx by industries and vehicles (Aderson and Dowing 2006). Considering the fluxes of both forms of inorganic N in the throughfall collected from our plots, the potential sources of N deposition can be the proximity of agricultural land commonly enriched with N fertilizers, intensive cattle husbandry in the forested area, and the wood and paper factory located in the vicinity.

Effects of N addition on the forest floor

Nutrient concentrations in the forest floor were influenced by N additions. The N treatments increased not only N but also P and K concentrations of the forest floor. Chen et al. (2019) also reported that P and N concentrations increased in response to N addition, with more P being mineralized, at least when the quality of the litter is sufficient. Although we did not measure the effects of nitrogen treatments on litter decomposition rates, Bragazza et al. (2012) claimed that mineral N addition reduced decomposition within the first year of their study. The increased C concentrations we found in the N treatments in the summer confirm this interpretation. Frey et al)2014) and Peng et al (2020) also report an N-induced reduction of decomposition rates. In spite of this, the mineralization of P that is organically bound in the litter can increase because the availability of N promotes the production of phosphatase enzymes (Saiya-Cork et al. 2002). We can speculate that this is also what happened in the forest floor of our study.

N addition and soil processes

The soil pH was higher in the dormant season compared to the growing season. It can be assumed that the pH decline in the topsoil was due to the increased biological activity prevailing during the growing season. The soil pH significantly decreased with increasing N additions. Similar results were reported in many studies from Europe, North America, and China (Lu et al. 2014; Verma and Sagar 2020). The addition of inorganic nitrogen caused soil acidification, which is coupled with displacing of base cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) from the soil exchange complexes, and leaching them, and thus reducing the soil buffering capaity (Matschonat and Matzner 1996). The observed decline in K concentrations over time in the four N treatments is an indication of this leaching process. The increased uptake by microorganisms and plants could not cause such a rapid decrease (Tomlinson 2003; Verma and Sagar 2020). When the plants absorb ammonium, the H^+ ion is released into the soil solution and causes soil acidification (Smith and Read 2008). According to the *mobile anion* concept (Reuss and Johnson 1986), the leaching of NO_3^- must be accompanied with base cations to maintain the charge equilibrium in the soil, resulting in higher concentrations of acid cations (Gundersen et al. 2006).

The concentration of P also decreased with the N treatments. In this case, an increased uptake by the trees can be responsible for this effect because the extractable pool of P is small compared to total soil P, which induces relatively rapid changes, especially where P availability is limited. This can explain the opposite effect that we observed between forest floor (more P) and the mineral soil (less available P). Similar results were reported by Luo et al. (2014) in an oak (*Quercus acutissima* Carruth.) plantation in China.

Soil organic C significantly increased in the high-N treatment. Soil C is in a balance between inputs (litterfall and decay fine root) and losses by respiration (Creamer et al. 2011) and by leaching of dissolved organic C (Findlay 2005). In our study, the observed decrease in soil microbial biomass has probably reduced soil CO_2 efflux to the atmosphere, which would elevate the amount of C in the soil (Bowden et al. 2004; Gundersen et al. 2012). Similar findings (increased soil C) were obtained in other N-addition experiments during the first year of N treatment (Nave et al. 2009). The results of Blanco et al. (2012), Ma et al. (2018), and Yan et al. (2018) also demonstrated that increasing N raises the soil C contents. The results of previous studies showed that the elevation in forest soil carbon content occurs because of two reasons:

first, an increase in forest productivity and litterfall by alleviating nitrogen limitation (Field et al. 2017), and second, a decreased SOM decomposition (Zang et al. 2016). In our case, because of the short duration of the N addition, a reduced decomposition rate is certainly the cause of the observed increase in soil C (Frey et al. 2014; Peng et al. 2020).

In the present study, microbial C showed a clear seasonality with a peak in the summer, when the temperature is maximal and there is still enough moisture from the winter precipitation, in agreement with seasonal variations reported in other studies (Noe et al. 2012; Hu et al. 2013). Such conditions are favorable for microbial biomass and activity, including the SOC decomposition processes. In this study, microbial C significantly decreased in the N treatments, which is similar to the results of Liu and Greaver (2010) and Zhang et al. (2019). Zhang et al. (2019) in their study in a *Pinus tabuliformis* Carr. forest showed that MBC decreased by almost one-fourth in their N treatments. This reduced microbial biomass may be due to NH_4NO_3 addition to the soil in the simulation process. Ammonium-nitrate is readily soluble, can rapidly react with an organic matter with low molecular weight, and stimulates the formation of stable compounds (Janssens et al. 2010). According to Baath et al. (1980), one of the reasons for the reduction of microbial biomass was a decrease in soil pH. Smolander et al. (1994) claimed that the soil organic matter changes with a decline of the soil pH, altering the structure of the microbial community. Wang et al. (2009) also indicated that soil acidity was an essential factor in reducing microbial biomass. In light of these findings, the reduced microbial biomass in our N treatments is undoubtedly explained to a large extent by the observed reduction in pH. In our N treatments, soil N increased much more than C, which means that the reduced C/N ratio (14%) of the substrate can also be the cause of the reduced decomposition rates (Feng et al. 2018).

Compared to the microbial biomass, the seasonality of the urease activity was delayed, with a peak in autumn. The N treatments had a significant positive effect on this enzymatic activity. Enzymes are N-rich compounds; therefore, their production is strongly dependent on the availability of N (Keeler et al. 2008). Intense stimulation of urease activity has also been the outcome of N deposition reported by Chai et al. 2019 and Saiya-Cork et al. 2002). This is likely due to an overall increase in soil N transformation. Indeed, even if there is an increase in N immobilization, some of the added N remains available for soil microbes and for all the biochemical transformations they mediate.

Conclusion

Our findings demonstrate that annual ambient N deposition (NH_4^+-N and NO_3^--N) fluxes in our study area (44 kg N ha−1 yr−1) were higher than that in the North America and Europe but were close to other hotspot regions in Asia. Our data were obtained with simulated deposition rates exceeding ambient rates, but still in the same order of magnitude. It is thus realistic to expect that ambient N deposition causes similar changes within a few years. Our findings suggest that, even in the short-term, N addition significantly affects the forest floor and soil properties (the forest floor and soil chemistry, microbial biomass C, and urease enzyme activity). Total N, urease, available P, available K, and MBC displayed the greatest variations in nitrogen treatments. Concerns about these results is especially high for Hyrcanian forests because they are of great ecological, cultural and economic values and have recently been considered as a UNESCO World Heritage Site. However, for future research, to assess the long-term effects on the forest ecosystems, long-term monitoring should include more aspects of the forest plantation characteristics, especially litter decomposition, soil enzyme activity, microbial and fungi community, and water relations. The detrimental effect of N deposition calls for measures to mitigate air pollution NOx (by traffic and industries) and NHy (by agriculture).

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Tables

Table 1. Results of repeated measures ANOVA of the nutrient concentrations in throughfall in N treatments and different seasons in oak (*Quercus castaneifolia*) plantation

Nutrient concentrations in throughfall	Different seasons			Nitrogen treatments			Different seasons× Nitrogen treatments		
	df	*F*-value	*P*	df	*F*-value	*P*	df	*F*-value	*P*
Throughfall (mm Month^{-1})	3	3878.411	**0.000**	3	0.159	0.923	9	0.077	1.000
pH (1:2.5 H_2O)	3	857.773	**0.000**	3	0.113	0.952	9	1.027	0.424
EC (ds/m)	3	263.456	**0.000**	3	0.004	1.000	9	0.075	0.995
NO_3^--N (mg L^{-1})	3	387.519	**0.000**	3	0.058	0.981	9	0.106	0.999
NH_4^+-N (mg L^{-1})	3	608.869	**0.000**	3	0.057	0.982	9	0.040	1.000
K^+ (mg L^{-1})	3	1323.732	**0.000**	3	0.015	0.997	9	0.024	1.000
PO_4^{3-} (mg L^{-1})	3	906.411	**0.000**	3	0.181	0.909	9	0.093	1.000

Bold number show significant differences ($P < 0.01$)

Table 2. Results of repeated measures ANOVA of the nutrient concentrations in the forest floor in N treatments and different seasons in oak (*Quercus castaneifolia*) plantation

Nutrient concentrations in the forest floor	Different seasons			Nitrogen treatments			Different seasons× Nitrogen treatments		
	df	*F*-value	*P*	df	*F*-value	*P*	df	*F*-value	*P*
N (%)	3	264.252	**0.000**	3	37.592	**0.000**	9	8.555	**0.000**
P (%)	3	49.947	**0.000**	3	3.306	**0.023**	9	0.995	0.441
K (%)	3	1672.708	**0.000**	3	2.722	**0.061**	9	3.659	**0.001**
C (%)	3	184.638	**0.000**	3	1.428	0.253	9	2.527	**0.012**

Bold number show significant differences ($P < 0.05$)

Table 3. Physical and chemical properties of the soil before applying N treatments in oak (*Quercus castaneifolia*) Plantation

Soil properties		Soil properties	
Moisture (%)	29.5	Clay (%)	13.3
Bulk density (g cm^{-3})	1.35	Lime (%)	2.85
Soil texture	Sandy-loamy	pH (1:2.5 H_2O)	6.1
Sand (%)	45.5	EC (ds/m)	0.49
Silt (%)	37.3	Organic C (%)	4.8

Table 4. Results of repeated measures ANOVA of the soil biochemistry in N treatments and different seasons in oak (*Quercus castaneifolia*) plantation

Nutrient concentrations in soil	Different seasons			Nitrogen treatments			Different seasons× Nitrogen treatments		
	df	*F*-value	*P*	df	*F*-value	*P*	df	*F*-value	*P*
pH (1:2.5 H_2O)	3	145.284	**0.000**	3	208.238	**0.000**	9	1.800	0.078
EC (ds/m)	3	134.150	**0.000**	3	22.901	**0.000**	9	1.671	0.107
Total N (%)	3	753.550	**0.000**	3	223.180	**0.000**	9	25.510	**0.000**
Available K (mg kg^{-1})	3	2151.520	**0.000**	3	1124.775	**0.000**	9	180.405	**0.000**
Available P (mg kg^{-1})	3	778.370	**0.000**	3	1645.314	**0.000**	9	322.760	**0.000**
Organic C (%)	3	30.039	**0.000**	3	40.529	**0.000**	9	7.757	**0.000**
MBC (mg kg^{-1})	3	64.796	**0.000**	3	23.536	**0.000**	9	5.349	**0.000**
Urease activity (µg N g^{-1}DM.2h^{-1})	3	112.609	**0.000**	3	13.876	**0.000**	9	1.758	0.081

Bold number show significant differences ($P < 0.01$)

Figures

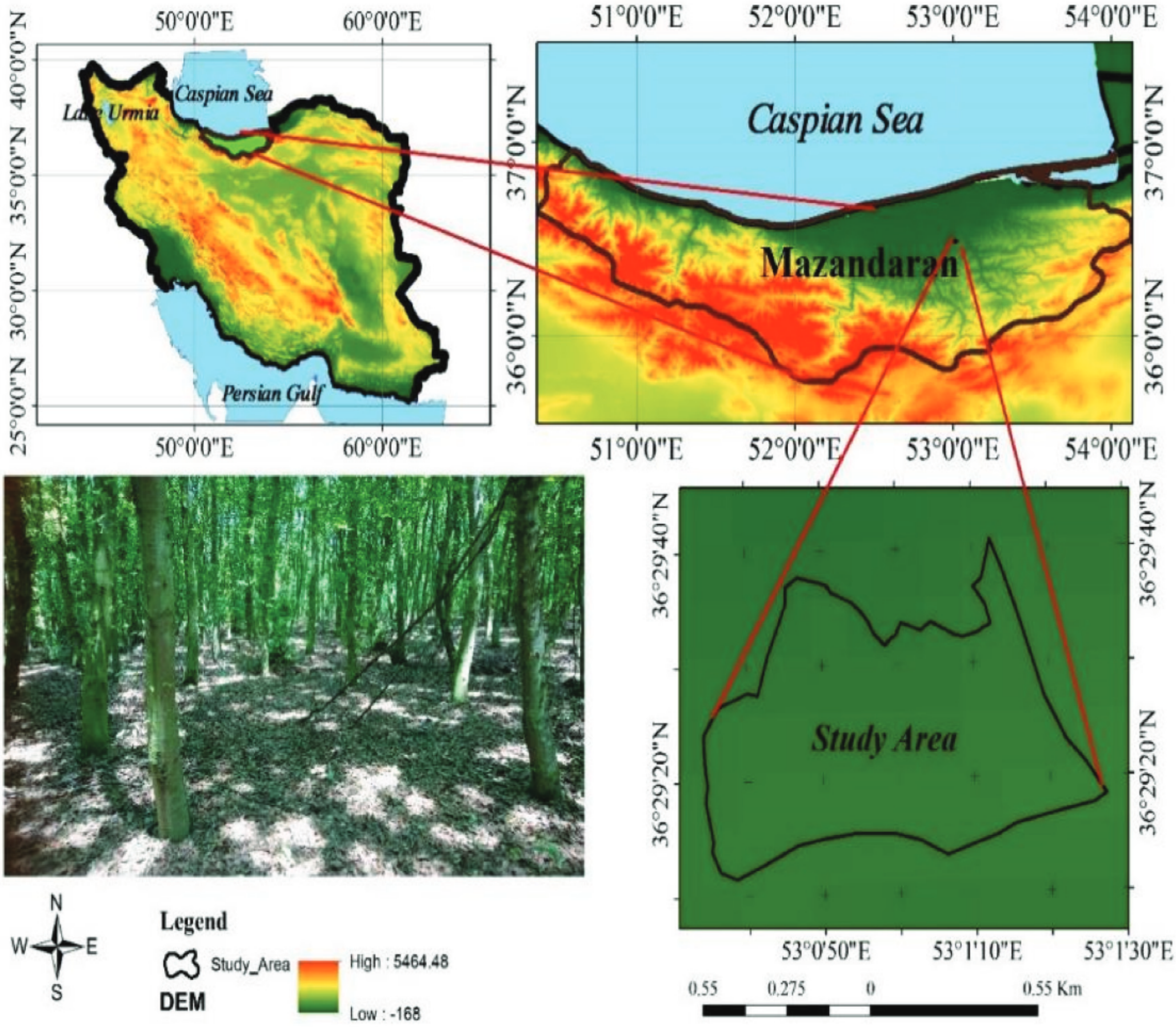

Fig. 1 Location of the study area, in oak (*Quercus castaneifolia*) Plantation, Sari City, Mazandaran Province, Iran. Base map and additional data from Forests, Rangelands and Watershed Management Organization (2021; http://www.frw.ir/02/En/default.aspx).

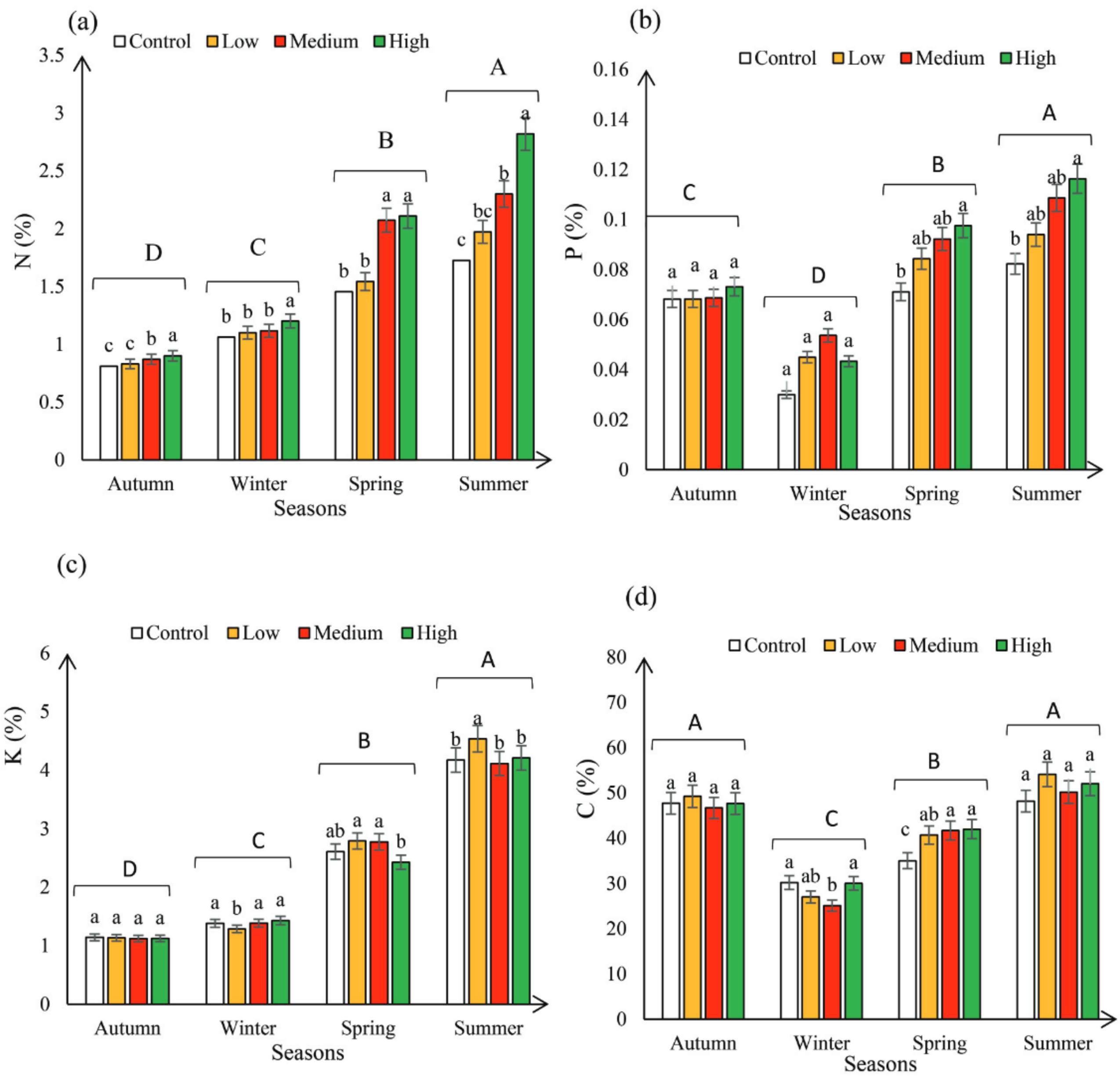

Fig. 2 The effects of different nitrogen deposition treatments on forest floor nutrient concentrations N (a), P (b), K (c), and C (d) in oak (*Quercus castaneifolia*) plantation (different lowercase letters indicate significance (*P* <0.05) differences between N treatments and different capital letters indicate significant (*P* <0.05) differences among seasons)

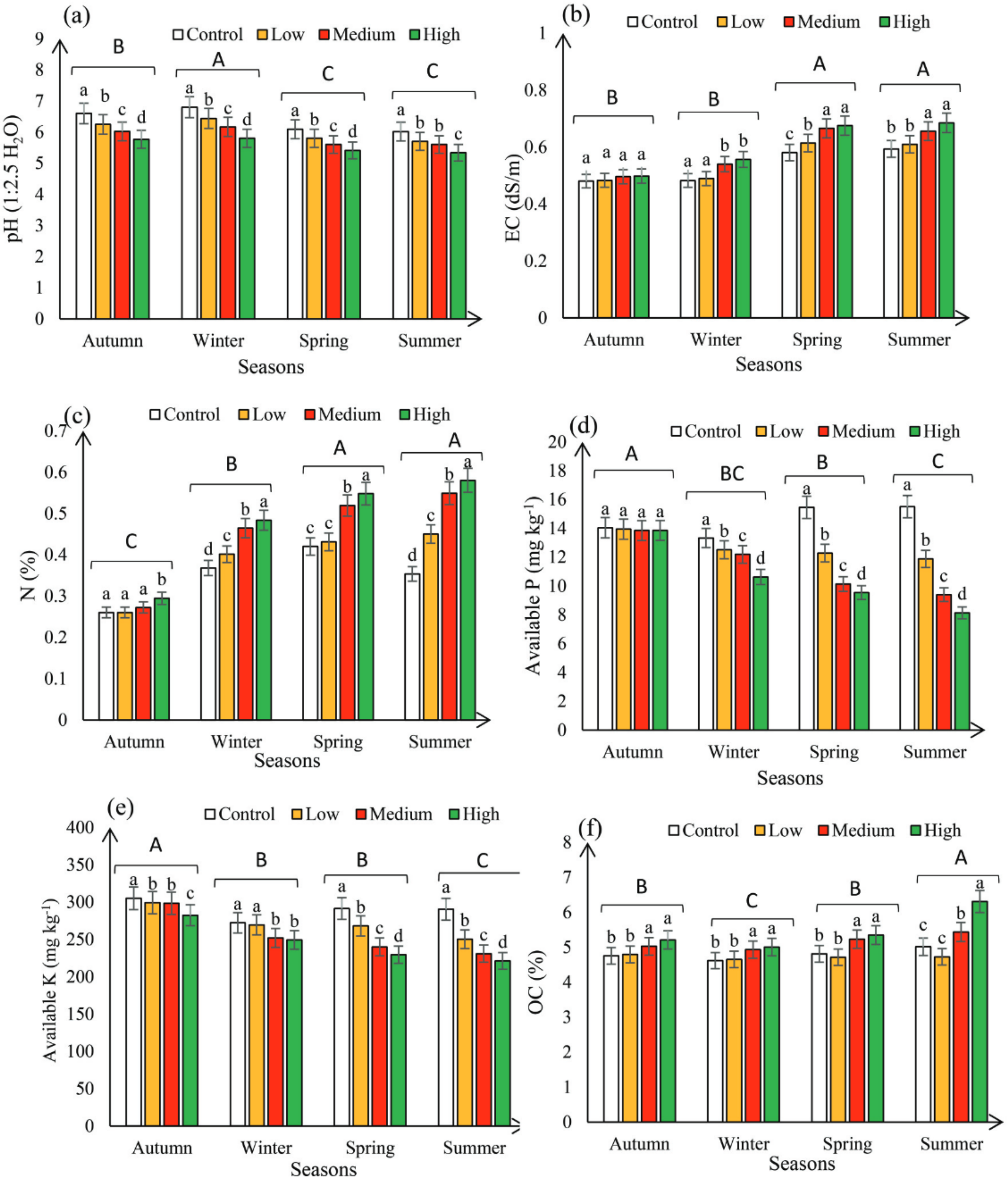

Fig. 3 The effects of different nitrogen deposition treatments on soil layer available K (a) OC (b) N (c) available P (d) pH (e) and EC (f) in oak (*Quercus castaneifolia*) plantation (different lowercase letters indicate significance (P <0.05) differences between N treatments and different capital letters indicate significant (P <0.05) differences among seasons)

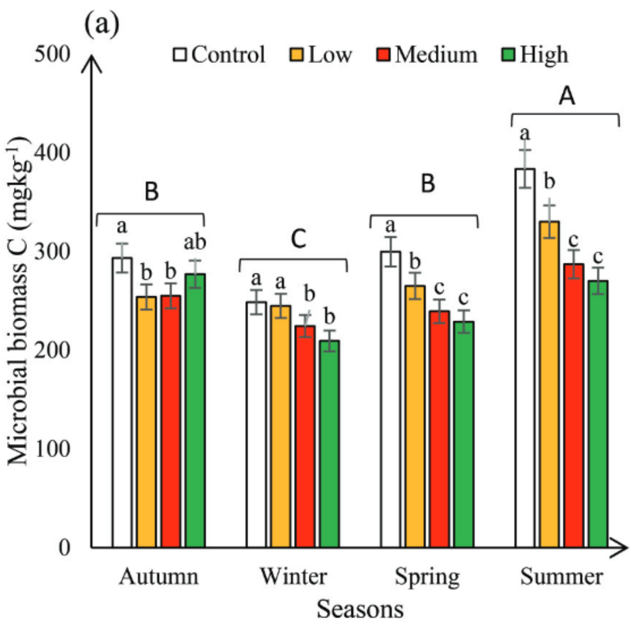

Fig. 4 The effects of different nitrogen deposition treatments on urease activity (a) and soil microbial biomass (b) in oak (*Quercus castaneifolia*) plantation (different lowercase letters indicate significance (*P* <0.05) differences between N treatments and different capital letters indicate significant (*P* <0.05) differences among seasons)

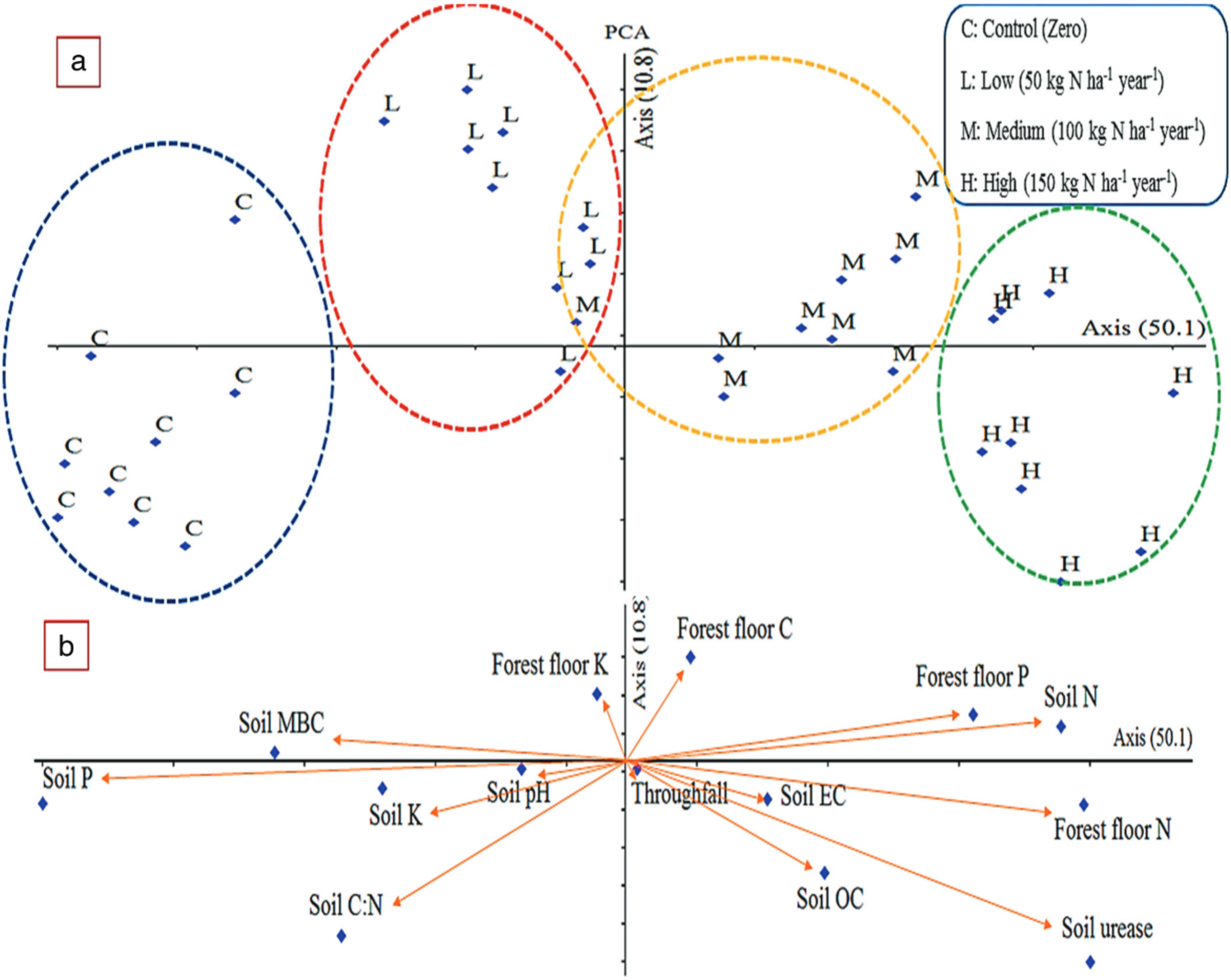

Fig. 5 PCA analysis of spatial distribution in N treatments (A) and forest floor and soil properties (B) (axis 1: eigenvalue = 7.01 with 50.1% of variance; axis 2: eigenvalue = 1.51 with 10.8% of variance) in oak (*Quercus castaneifolia*) plantation

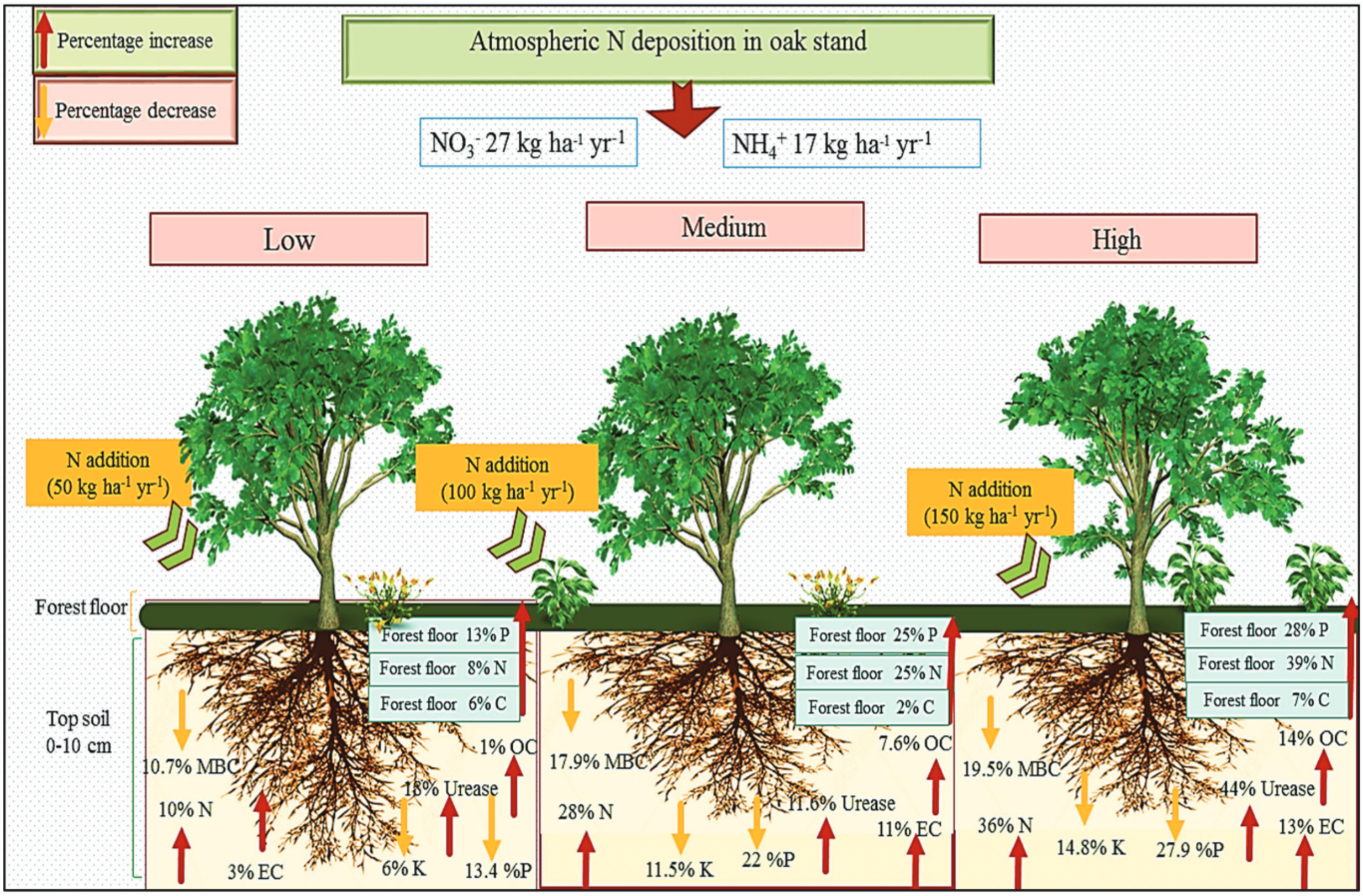

Fig. 6 Diagram summarizing of the relative changes on soil and forest floor chemical properties under N treatments in oak (*Quercus castaneifolia*) plantation