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Nitrogen accumulation in forest floors with introduced *Pinus pinea* and *Pinus pinaster* in dune site

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Abstract Introduced stone pine (*Pinus pinea* L.) and maritime pine (*Pinus pinaster* Aiton) stands were sampled 60 years after plantation in Istanbul-Durusu (Terkos), Turkey. Sampling was conducted at four different developmental stages (mean diameter of trees at 1.3-m height (DBH) in stands: SDF=<8 cm, MDF=8–20 cm, LDF=20–36 cm, and UDF>36 cm), with 15 replicated sample plots for each species and developmental stage, for a total of 120 sample plots. The forest floor was sampled in 5 replications in each sample plot. The forest floor samples were divided into two layers: L+F (litter+fermentation) and H (humus), and the oven dry mass and nitrogen (N) content were determined. As a result, the masses of the total forest floor and of both layers were significantly different among the development stages with an increasing trend in maritime pine stands despite no significant difference found in the mass of the humus layer in stone pine stands. However, total forest floor accumulation significantly increased with the development stage (28–60 t/ha in stone pine and 17–64 t/ha in maritime pine). In both species, the N concentrations in the forest floor layers differed significantly among the development stages, and the N concentrations tended to increase as the development stage increased in the L+F layer, whereas a fluctuating trend was observed in the H layer. Although the N content of the humus layer of stone pine did not show a significant difference among the development stages, the N content in the total forest floor was determined to be 0.1–0.5 t/ha in maritime pine and 0.2–0.5 t/ha in stone pine. The relationship between the mean stand DBH and the N stock of the total forest floor was determined to have a higher correlation in maritime pine (R^2=0.8) than stone pine (R^2=0.4). In conclusion, the nitrogen concentrations and nitrogen contents of the forest floor were remarkably different in stands introduced with different tree species, indicating the accumulation of forest floor nitrogen.

Highlights
• total forest floor accumulation in stone pine and maritime pine significantly increased with the development stage.
• N concentrations in the forest floor layers differed significantly among the development stages.
• N concentrations tended to increase as the development stage increased in the L + F layer.
• N contents of forest floor layers increased with the development stages except N content of the humus layer in stone pine.

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Introduction

Sand dunes constitute approximately 33% of the total coastal environments in the world (Malavasi et al., 2013). Destructive water and wind erosion of dunes and the movement of sand particles have several negative effects on the environment (Niu et al., 2015). The mobilization of unbound sand grains over long distances threatens many ecosystems, such as settlements, freshwater resources, and agricultural areas. Introducing suitable tree or plant species and revegetating dunes are the most valid methods for preventing sand movement and restoring dune ecosystems (Moradi et al., 2017; Su et al., 2005). Introduced plant cover and tree plantations prevent erosion and increase organic carbon (Garcia-Franco et al., 2014), nutrients (especially nitrogen), and the sequestration of CO_2 (Hu et al., 2015).

Previous studies have reported that introducing different tree species creates forest floors with different physical and chemical characteristics; these differences substantially affect soil properties (Abdalmoula et al., 2019; Bengtson et al., 2006; Makineci et al., 2015) and the sustainability and productivity of ecosystems (Fisher & Binkley, 2012). In particular, nutrient fluxes, particularly of carbon and nitrogen, from forest floors are related to the quality of the forest floors (Fisher & Binkley, 2012; Fonseca & Figueiredo, 2018; Park, 2015). Forest floors generally have a multifarious morphology and can be partitioned into various layers representing the successive stages of decomposition; additionally, the N availability of a forest floor is very important for decomposition process (Thomas & Prescott, 2000). For this reason, plantations and accretions of organic matter have specific priorities in dune restorations, as dunes have poor water and nutrient capacities (Drius et al., 2016; Su & Zhao, 2003). Forest floors are also very important organic matter reserves in afforestation sites, especially in pine plantations (Li et al., 2011), and the sampling of successive developmental stages of stands is generally the most efficient method to evaluate the effects of plantations on forest floors (De Simon et al., 2012; Ozdemir et al., 2013; Tolunay et al., 2017).

Nitrogen is also the most effective nutrient element in forest ecosystems, along with the importance of organic matter, and nitrogen deficiency limits ecosystem productivity (Castro-Díez et al., 2012; Vesterdal et al., 2008). Furthermore, the availability and budget of nitrogen in forest ecosystems have always been of increasing interest and importance (Aber et al., 1995; Grofmann et al., 2001). Nitrogen availability in the restoration process is one of the pioneering criteria for the restoration and productivity of the ecosystem, in addition to its limiting effect that affects success in the restoration and plantation of sand dunes, which are particularly poor in nutrients (Li et al., 2013). Despite this remarkable importance of nitrogen, the change in the soil after afforestation is still uncertain (Hoogmoed et al., 2012). On the other hand, knowledge on the effects of planted tree species on nitrogen stocks is still scarce (Fonseca & Figueiredo, 2018; Jasińska et al., 2020).

Many ecological restoration studies have been conducted in Turkey since 1950 to protect ecosystems against dunes threatening agricultural lands, settlements and drinking water reserves (Atay, 1964; Büyükyıldırım, 1961; Kilci et al., 2010; Öztürk, 1991; Saatcioglu et al., 1978; Tüfekçi, 1992; Yaktı, 2003; Yesilkaya & Neyisci, 1991). One of these restoration projects was conducted in the Istanbul-Durusu (Terkos) coastal dune, which is the research area of the present study and is a very important drinking water reservoir for Istanbul with a population over 15 million. Durusu Lake is the oldest drinking water source (established in 1883); it is currently the second most essential water reservoir of the Istanbul metropolitan city and has maintained its importance over years (Abdalmoula et al., 2019; Tolunay et al., 2017). Similar problems for plantations and dunes, such as severe wind erosion, poor water and nutrients and the selection of suitable species, are also common in this area. The restoration process in the Durusu dune area has been launched to prevent sand filling in Durusu Lake and erosion (Abdalmoula et al., 2019; Tolunay et al., 2017). Atay (1964), Kantarcı et al. (1973), and Saatcioglu et al. (1978) stated no organic matter accumulation or nitrogen in Durusu dune before afforestation studies. Different tree species have been tested with preliminary studies, and these tests showed that stone pine and maritime pine have been determined to be successful species for afforestation, and both tree species have been used exclusively since the end of the 1950s (Atay, 1972, 1981; Saracoglu & Bozkus, 1996).

The main purpose of this study was to investigate the contents of nitrogen stored in the forest floor in different developmental stages of introduced stone pine and maritime pine in the dune area of Istanbul-Durusu (Terkos) after approximately 60 years. Under both tree species, the nitrogen contents of the forest floor were determined and compared in the development stages and correlated with the mean DBHs of the stands. Thus, it was hypothesized that in dune maritime pine and stope pine plantations, (1) mass, nitrogen concentration, and nitrogen content of forest floor are significantly different in particular stand development stages and (2) nitrogen contents of different forest floor layers significantly vary with mean tree DBH of stand.

Material and method

Research site

The study was conducted in the Durusu (Terkos) coastal dune area in Istanbul-Turkey located on the western coast of the Black Sea (41°19′ 04″–41° 22′ 28″ north latitudes and 28° 29′ 28″–28° 39′ 44″ east longitudes). The surface area of the dune is approximately 3350 ha, the average slope gradients range from 0 to 15%, the southwest is the main aspect of land, and the average altitude is approximately 20 m asl. The annual mean rainfall is 1088.8 mm, and the annual mean temperature is 12.7 °C (Abdalmoula, 2017). The geological parent material of the area is coastal sand material. Since the research area is generally flat or nearly flat, it is quite homogeneous in terms of site characteristics (parent material, slope, aspect, and altitude). Small sand grains can be easily moved by wind erosion, and their available water capacity and organic carbon content are very poor (Abdalmoula et al., 2019; Kantarcı et al., 1973). The calcium carbonate ($CaCO_2$) content is approximately 2–12%, and the acidity is between slightly acidic and slightly alkali (6–8 pH) (Abdalmoula et al., 2019).

The first plantations on Durusu sand dune started in 1959; initially, the first experiments with different species started this year in an area of approximately 5 ha (Atay, 1964). Stone pine (*Pinus pinea* L.) and maritime pine (*Pinus pinaster* Ait.) species were determined to be more successful in 1963, and afforestation was made largely using these species later. Currently, maritime pine plantations have a 1674 ha area, and stone pine plantations cover a 371 ha area on the Durusu sand dune (Abdalmoula, 2017; Abdalmoula et al., 2019; Tolunay et al., 2017).

Sampling

The classification of the Turkish General Directorate of Forestry was used for the stand development stages. Accordingly, the development stages were small diameter forest (SDF): DBH = 0–8 cm, medium diameter forest (MDF): DBH = 8–20 cm, large diameter forest (LDF): DBH = 20–36 cm, and upper large diameter forest (UDF): DBH > 36 cm. Each of the different development stages of the maritime and stone pine plantations was sampled with 15 replications; thus, the total number of sample plots (20 × 20 m) was 120. Before sampling, the forest floor in each sample plot, the number, ages, DBHs and heights of trees were measured to determine the basic stand characteristics (Table 1) (Abdalmoula et al., 2019).

The forest floor samples (each 25 × 25 cm in size) were taken in 5 replications in each plot by collecting all the forest floors on the mineral soil layer. A total of 600 (2 species × 4 development stages ×15 replicated sample plots × 5 replicated forest floor samples) forest floor samples were collected, as described below, by separating them into litter + fermentation (L+F) and humus (H) layers, thus the total number of forest floor samples was 1200 (Abdalmoula, 2017; Abdalmoula et al., 2019).

Table 1 Stand characteristics of sample plots (Abdalmoula et al., 2019)

Maritime pine (*Pinus pinaster* Aiton.)					
Development Stages	Age	Density (tree/ha)	$D_{0.3\ m}$ (cm)	DBH ($D_{1.3\ m}$) (cm)	Height (m)
SDF	28 ± 2	3160 ± 354	9.5 ± 0.4	4.5 ± 0.4	2.4 ± 0.2
MDF	31 ± 2	1663 ± 149	19.6 ± 0.8	15.5 ± 0.7	9.1 ± 0.6
LDF	42 ± 1	920 ± 57	29.2 ± 0.8	24.5 ± 0.8	15.0 ± 0.4
UDF	44 ± 0	681 ± 29	37.2 ± 0.8	32.3 ± 0.6	19.5 ± 0.6
Stone pine (*Pinus pinea* L.)					
SDF	35 ± 4	2856 ± 548	10.1 ± 0.7	5.0 ± 0.5	2.4 ± 0.2
MDF	35 ± 2	1385 ± 245	19.0 ± 0.7	14.5 ± 0.6	5.5 ± 0.3
LDF	37 ± 2	671 ± 104	28.9 ± 1.3	22.9 ± 0.8	8.3 ± 0.5
UDF	40 ± 3	213 ± 25	44.1 ± 0.9	36.9 ± 0.9	12.7 ± 0.3

± standard error (DBH in which small-diameter forest (*SDF*) 0–8 cm medium-diameter forest (*MDF*) 8–20 cm large-diameter forest (*LDF*) 20–36 cm and upper large-diameter forest (*UDF*) > 36 cm)

Laboratory analyses

The air-dried forest floor samples were divided into L+F and H layers by using sieves with a 1-mm screen size. The subsamples were dried at 70 °C to detect oven dry masses of both layers. A composite forest floor sample was prepared by taking equal amounts from each replicated sample for the same sample plot. Nitrogen analyses were performed on a LECO Truspec 2000 analyzer. The nitrogen contents in the forest floor layers per unit area were found by multiplying the masses of the layers by the nitrogen concentrations (%) (Abdalmoula, 2017; Abdalmoula et al., 2019).

Statistical evaluation

The analysis of variance (ANOVA) was used to test the differences between nitrogen concentration and contents in forest floor according to the stand development stages. The normality of distribution was checked with Kolmogorov–Smirnov test. Variables with a normal distribution and 95% confidence were also subjected to ANOVA, and variables not presenting a normal distribution were transformed to a normal distribution with a logarithmic transformation. Duncan's post hoc test was performed at the $\alpha = 0.05$ significance level if the differences in the arithmetic means were determined to be statistically significant according to the ANOVA results. A simple linear regression analysis was applied to obtain the estimation value of the relationship (DBH) of the nitrogen content in the different forest floor layers and the stand DBH. The SPSS 21.0 software was used for all analyses.

Results

All forest floor characteristics in the maritime pine plots were found to be significantly different among the development stages. The change in the forest floor mass according to the stand development stages increased towards the UDF development stage in both the L+F and the H layers. In other words, there is an accumulation of forest floor over time (Abdalmoula et al., 2019). The nitrogen concentrations of forest floor layers ranged from 0.6% and 0.94%, and the highest nitrogen concentration in both the L+F and H layers was in the LDF development stage. Because of the significant change in the mass of forest floor layers and total forest floor mass as the stand development stage increased, the nitrogen contents also changed significantly among the development stages and increased as the development stage increased (0.1–0.5 t/ha) (Table 2). The regression coefficients between the stand DBHs and nitrogen contents of the total forest floor were $R^2 = 0.76$ in the L+F layer, $R^2 = 0.71$ in the H layer, and $R^2 = 0.79$ in the total forest floor (Fig. 1).

Although the total forest floor mass and L+F layer mass differed significantly among the development stages in stone pine plots, the MDF, LDF and UDF development stages had similar mass values; the value of the SDF development stage differed. The highest total forest floor mass was in the MDF stage, and there was no increasing trend with the development stage despite the total forest floor mass increasing approximately twice (28–60 t/ha) compared to the youngest development stage (SDF).

Table 2 Nitrogen in forest floor of maritime pine plantations on Durusu-Istanbul sand dune

Forest floor properties	Development stages SDF-MP	MDF-MP	LDF-MP	UDF-MP	P
L+F layer					
Mass (t/ha)	10.2 a ± 1.2	18.0 b ± 1.4	28.2 c ± 2.3	32.6 c ± 1.9	0.000
N (%)	0.65 a ± 0.04	0.76 b ± 0.03	0.94 c ± 0.04	0.90 c ± 0.03	0.000
N (t/ha)	0.07 a ± 0.01	0.14 b ± 0.01	0.27 c ± 0.03	0.29 c ± 0.02	0.000
H layer					
Mass (t/ha)	6.6 a ± 1.0	13.9 ab ± 1.8	20.0 b ± 3.3	31.0 c ± 5.0	0.000
N (%)	0.60 a ± 0.03	0.74 a ± 0.04	0.82 a ± 0.03	0.76 ab ± 0.07	0.016
N (t/ha)	0.04 a ± 0.01	0.10 b ± 0.01	0.16 c ± 0.03	0.20 c ± 0.02	0.000
Total forest floor					
Mass (t/ha)	16.8 a ± 2.0	31.9 b ± 2.4	48.2 c ± 5.2	63.6 d ± 6.2	0.000
N (t/ha)	0.10 a ± 0.01	0.24 b ± 0.02	0.44 c ± 0.05	0.50 c ± 0.03	0.000

± standard error (DBH in which small-diameter forest (*SDF*) 0–8 cm medium-diameter forest (*MDF*) 8–20 cm large-diameter forest (*LDF*) 20–36 cm and upper large-diameter forest (*UDF*) > 36 cm) MP maritime pine masses of forest floor layers and total forest floor were also presented in Abdalmoula et al. (2019)

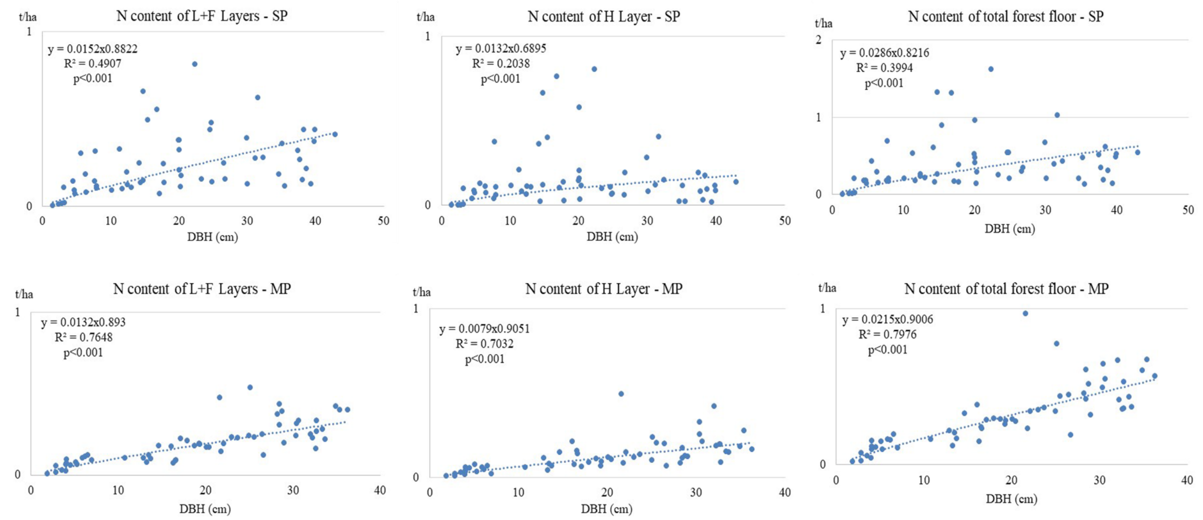

Fig. 1 The relationship between the nitrogen (N) content in the different forest floor layers and the stand DBH in simple linear regression analysis, (*SP*) stone pine and (*MP*) maritime pine

The mass of the humus layer did not differ significantly in terms of the development stages (Table 3). The nitrogen concentrations of both layers were significantly different in different development stages: the L + F layer had a significantly increasing trend (0.78–0.99%) from SDF to UDF; however, no clear tendency was observed in the H layer (0.46–0.72%) (Table 3). The nitrogen contents significantly increased from SDF (0.11 t/ha) to UDF (0.3 t/ha) in the L + F layer and were between 0.19 t/ha (SDF) and 0.5 t/ha (MDF) in the total forest floor. However, the nitrogen content in the H layer did not change significantly in the development stages (Table 3). The regression coefficients between the stand DBHs and nitrogen contents of the total forest floor, L + F layer and H layer were determined to be $R^2 = 0.39$, $R^2 = 0.49$, and $R^2 = 0.21$, respectively (Fig. 1).

Table 3 Nitrogen in forest floor of stone pine plantations on Durusu-Istanbul sand dune

± standard error (DBH in which small-diameter forest (*SDF*) 0–8 cm medium-diameter forest (*MDF*) 8–20 cm large-diameter forest (*LDF*) 20–36 cm and upper large-diameter forest (*UDF*) > 36 cm) (*SP*) stone pine, masses of forest floor layers and total forest floor were also presented in Abdalmoula et al. (2019)

Forest floor properties	Development stages				P
	SDF-SP	MDF-SP	LDF-SP	UDF-SP	
L + F layer					
Mass (t/ha)	15.4 a ± 3.6	26.4 ab ± 5.0	30.7 b ± 4.4	30.1 b ± 3.1	0.048
N (%)	0.78 a ± 0.18	0.89 b ± 0.14	0.99 b ± 0.15	0.99 b ± 0.14	0.000
N (t/ha)	0.11 a ± 0.10	0.24 b ± 0.19	0.30 b ± 0.19	0.30 b ± 0.14	0.004
H layer					
Mass (t/ha)	12.4 a ± 2.9	29.2 a ± 6.4	28.9 a ± 6.2	26.6 a ± 4.4	0.092
N (%)	0.63 b ± 0.15	0.72 b ± 0.17	0.68 b ± 0.23	0.46 a ± 0.11	0.000
N (t/ha)	0.08 a ± 0.09	0.22 a ± 0.23	0.20 a ± 0.21	0.12 a ± 0.10	0.093
Total forest floor					
Mass (t/ha)	27.8 a ± 6.2	55.6 b ± 11.5	59.6 b ± 10.4	56.7 b ± 7.3	0.022
N (t/ha)	0.19 a ± 0.18	0.46 b ± 0.40	0.50 b ± 0.37	0.42 b ± 0.23	0.040

Discussion

In general, the masses of layers and the total forest floor increased in both species with increasing development stages. Changes in the masses of forest floor layers were also documented and evaluated in detail by Abdalmoula et al. (2019). Similar results on the changes in forest floor masses for maritime pine and stone pine have also been previously reported (Kantarcı, 2000; Ozdemir et al., 2013; Sever & Makineci, 2009; Tolunay et al., 2017), indicating the accumulation of the forest floor under these species. In particular, the accumulation of the forest floor was determined in stone pine (Keskin & Makineci, 2009) and maritime pine (Sever & Makineci, 2009) afforestation 18 years after installation on an old coal mine reclamation site, which is close to the research area of the present study and has similar local site conditions. In addition, some identical results have also been obtained in a plantation of stone pine on a clay mine reclamation site (Karatepe et al., 2020) and in a stone pine plantation on a sand dune in Tarsus-Turkey (Kizildag et al., 2012).

The accumulation of the forest floor is generally confirmed under coniferous species (Bargali et al., 2015; Fonseca & Figueiredo, 2018; Kantarcı, 2000), and enormous amounts of dead organic materials slowly decompose because the decomposition process is controlled by the chemistry of the forest floor as a determining factor (Guendehou et al., 2014; Kantarcı, 2000; Kooch et al., 2017). Low activity of decomposer organisms may also have an impact on reluctant decomposition and forest floor accumulation. Ripley and Pammenter (2008) stated that the reason for the low plant biomass in sand dunes is insufficient resource availability. In addition, on sand dunes, slow-insufficient decomposition of the forest floor might also have an effect on insufficient resource availability (Koijman, 2008). Furthermore, the poor water holding capacities and low nutrients of sand dunes are not desirable conditions for decomposer soil organisms and decrease their biological activity. Similarly, Moradi et al. (2017) indicated that even under a nitrogen-fixing species (*Prosopis juliflora*), there is little decomposition and little organic matter leaching into the dune.

The nitrogen concentrations of the forest floor layers of both species show similar results to those found in the literature in various dune areas with different afforestation and vegetation applications (Li et al., 2017; Zhang et al., 2013). Fonseca and Figueiredo (2018) reported N concentrations between 0.54% and 1.4% for *Pinus pinaster* in different forest floor layers and components. Ribbons et al. (2016) found the N concentrations in the forest floor of four different coniferous species (cedar, fir, hemlock, and spruce) to be between 1.05 and 1.35%. Thomas and Prescott (2000) reported the mean N concentration of forest floor in adjacent plantations of different tree species as 1.65%. Jerabkova et al. (2006) found the mean N concentration of forest floors in coniferous forests to be 1.219% in Canada.

There are different results in the literature regarding the changes in forest floor N concentrations in development stages. For example, Mroz et al. (1985) reported that the N concentration of the forest floor has a decreasing trend in the incipient development stages of plantations. Many researchers have also emphasized that the N increases of forest floors and soils, together with carbon, increase with stand development processes (Abdalmoula et al., 2019; Makineci et al., 2015; Tolunay et al., 2017; Yu & Jia, 2014; Zhou et al., 2011). As determined in the present study, with increasing N concentrations, especially in the L + F layers, in the developmental stages of plantations, the increase in N concentration during the decomposition period is a generally occurring phenomenon in coniferous stands (Bargali et al., 2015; Trum et al., 2011).

The N content of the total forest floor increased with the stand development stage and showed a significant correlation with the stand DBH in the present study. Similarly, Goh and Heng (1987) stated that N contents increased in different components of the forest floor with increasing plantation age. Nitrogen is immobilized in the forest floor stored on mineral soil in coniferous stands. In these stands, the accumulation of organic material is favored due to undesirable litter quality for decomposers, which in turn changes the rate of decomposition and causes the accumulation of forest floor on soil (Fonseca & Figueiredo, 2018); thus, nitrogen content also increases with stand development. Additionally, increased forest floor N concentrations at development stages facilitate the accumulation of nitrogen content in the forest floor. In particular for stone pine and maritime pine, similar results were reported for the accumulation of mass and N content of forest floor by Keskin and Makineci (2009) and Karatepe et al. (2020) (for stone pine) and by Sever and Makineci (2009); Ozdemir et al. (2013); Kahyaoğlu and Güvendi (2020) (for maritime pine). Additionally, especially for sand dunes, Li et al. (2013) reported that the soil N (inorganic + organic) contents were 1.4 and 1.5 times higher than those of the control area under *Pinus sylvestris* var. Mongolica Litv. plantations at the ages of 28 and 38, respectively, on active sand dunes. Kizildag et al. (2012) indicated a similar situation in both maritime pine and stone pine, and Polat (2010) reported the same for stone pine on dune areas in Turkey.

Despite the influence of topographic position was not taken into account in present study, Sewerniak and Puchałka (2020) shown that particular topographic positions differ in the N-fertility index, which is a proxy for nitrogen content, and this allows for the planning of species-diverse stands in the dunes. On the other hand, Jasińska et al. (2020) did not show statistically significant differences in the amount of nitrogen in the topsoil between the northern and southern slopes.

In conclusion, the nitrogen concentrations and contents of forest floor are remarkably different between stands introduced with maritime pine and stone pine species on the Durusu sand dunes, indicating the accumulation of forest floor nitrogen contents. However, the present study has some limitations: only one sampling was performed 60 years after introducing the species and installing the stands. Future research should also take into account the seasonal variability of the content of nitrogen in forest floor (see Jasińska et al., 2020) and the influence of the topographic position, as this determines the microclimate at different dune exposures and topographic positions. For the stability of forests on dunes, it is worth considering admixing deciduous trees in given depressions and on the northern slopes because the pine monocultures that are planted in dunes are susceptible to wildfires. The admixture of deciduous trees on the northern slopes and in the dunes depressions should reduce this risk and have a positive effect on biodiversity (Sewerniak & Puchałka, 2020). Also, subsequent sampling and testing of the decomposition processes of forest floors can provide more detailed results for ecological restoration processes and ecosystem improvements.

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