



# Effect of nitrogen addition on soil CO<sub>2</sub> efflux and fine root biomass in maple monocultures of the hyrcanian region

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Received: 5 October 2020 / Accepted: 24 February 2021 / Published online: 23 March 2021  
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## Abstract

• **Key message** Nitrogen (N) addition (10 and 15 g N m<sup>-2</sup> year<sup>-1</sup> as dissolved NH<sub>4</sub>NO<sub>3</sub>) significantly increased the CO<sub>2</sub> efflux from the forest soil and the fine root biomass in a maple (*Acer velutinum* Bioss.) plantation. Following a seasonal pattern, soil CO<sub>2</sub> efflux showed an exponential relationship with the fine root biomass and soil temperature.

• **Context** The effect of increased atmospheric Nitrogen (N) deposition on forest soil CO<sub>2</sub> efflux is still unclear in the Hyrcanian forests and has received considerable attention in the context of global climate change.

• **Aims** Aims of this study were to determine how soil CO<sub>2</sub> efflux and fine root biomass change after N addition in a maple (*Acer velutinum* Bioss.) plantation.

• **Methods** Since the wet N deposition in these areas is 3–5 g N m<sup>-2</sup> year<sup>-1</sup>, four treatments including N1 (5 g N m<sup>-2</sup> year<sup>-1</sup>), N2 (10 g N m<sup>-2</sup> year<sup>-1</sup>), N3 (15 g N m<sup>-2</sup> year<sup>-1</sup>), and N0 (control) were selected. Twelve plots (10 × 20 m) were established, and a NH<sub>4</sub>NO<sub>3</sub> solution was sprayed monthly below the trees' canopy for 1 year. Soil temperature, moisture, and soil CO<sub>2</sub> efflux were measured monthly with static dark closed chambers. Fine root biomass was seasonally measured by soil sampling at the same depth.

• **Results** Soil temperature, moisture, and soil CO<sub>2</sub> efflux were affected by different levels of N addition. Soil CO<sub>2</sub> efflux significantly increased with N addition, and N3 displayed the highest rate (174 ± 16.1 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>). Fine root biomass increased significantly in N3.

• **Conclusion** The predicted levels of N deposition in such plantations will probably lead to enhanced CO<sub>2</sub> efflux from soils in reforested areas close to industrial sites in the Hyrcanian forest.

**Keywords** CO<sub>2</sub> efflux · Enhanced nitrogen addition · Hyrcanian maple forest · Fine root growth

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**Handling Editor:** Shuguang (Leo) Liu

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**Contributions of the co-authors** MT Investigation, field sampling and writing original draft; SMH designed the study, supervision, review, and editing the manuscript; HJ data preparation and analysis; NL the interpretation and discussion of the results, review, and editing the manuscript. MT data analysis and editing the manuscript.

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## 1 Introduction

Human activities such as consumption of fossil fuels, disturbance of forests, land-use change, and agricultural fertilization have substantially increased the rate of atmospheric Nitrogen deposition, specifically under the form of N oxides (NO<sub>x</sub>) (Galloway et al. 2004; Dentener et al. 2006; Bobbink et al. 2010; Song et al. 2017). Although atmospheric nitrogen deposition has positive effect such as increased tree growth in N-limited forests (Wardle et al. 2004; Li et al. 2019; Liu et al. 2020), it has many negative ecological effects on terrestrial and aquatic ecosystems, such as eutrophication and loss of biodiversity (Maskell et al. 2010; Janssens et al. 2010). Atmospheric deposition of N is already a serious problem in Europe and the USA; in addition, the fastest rate of increase in N deposition has been documented in the developing industrial regions of Asia (Mo et al. 2006;

Zhu et al. 2015a, b; Jia et al. 2016; Zheng et al. 2018). Moreover, atmospheric N deposition is predicted to increase from 25–40 Tg per year to 60–100 Tg per year until the end of this century at global scale (Lamarque 2005; Zhang et al. 2014; Wei et al. 2020). Therefore, concern about the ecological effects of elevated N deposition on terrestrial ecosystems is currently increasing (Magill et al. 2004), especially on forest ecosystems (Keenan et al. 2015).

Chronic N deposition has many negative effects on forest ecosystems globally (Carter et al. 2017; Deng et al. 2018; Schulte-Uebbing and De Vries 2018; Shi et al. 2018) including the biodiversity (Riofrío-Dillon et al. 2017; Vitousek et al. 1997), forest soils (e.g. decrease in pH and electrical conductivity (EC)), foliar chemistry, and soil biomass (Lupi et al. 2013; Novotný et al. 2016; Zhang et al. 2018; Gentilesca et al. 2018; Tafazoli et al. 2019). Considering that forests are known as a sink for carbon (C) with an important role in the global C cycle (Naik et al. 2018), the impacts of increased N inputs on the forest soil organic carbon (SOC) dynamics and cycle are of great concern (Janssens et al. 2010; Wieder et al. 2015; Chen et al. 2018). Since CO<sub>2</sub> efflux from forest soils is through plant root respiration, rhizomicrobial respiration, and soil organic matter decomposition (Jiang et al. 2010), N deposition can alter the rates of microbial N and C turnover, and thus affect the CO<sub>2</sub> efflux from forests' soil (Liu and Greaver 2009).

Due to differences in vegetation and soil properties in different forests, the responses of soil CO<sub>2</sub> efflux and fine root biomass to N deposition may include promotion (Cleveland and Townsend 2006; Peng et al. 2011; Tu et al. 2011), decrease (Janssens et al. 2010; Ramirez et al. 2010), and no effect (Allison et al. 2008; Samuelson et al. 2009). To date, it is not clear how atmospheric N deposition affects the soil CO<sub>2</sub> efflux and fine root biomass in the Hyrcanian Forests; therefore, understanding how N additions alter these two factors still remains an important scientific challenge (Tu et al. 2013).

Iran, especially the northern part where the Hyrcanian forests are located, is experiencing the release of a great amount of N into the atmosphere. The main sources include developing industrial regions, combustion of fossil fuels, and use of artificial fertilizers (Salahi et al. 2014; Nobakht et al. 2018). The Hyrcanian forests, which were recently registered as a UNESCO World Heritage Site, are a green belt on the northern slopes of Alborz Mountains and cover the southern coasts of the Caspian Sea (Sagheb-Talebi et al. 2014). These forests are mixed deciduous and temperate forests; they appear to be very similar to the broadleaf forests of central Europe, northern Turkey, and the Caucasus (Sagheb-Talebi et al. 2014). Unfortunately, degradation of Hyrcanian forests along with N deposition not only may have some negative local effect but also can have significant effects on the global C

cycle. Since Hyrcanian forests are N-limited, N deposition can stimulate primary production and sequestration of C in these ecosystems (De Vries et al. 2006; Sutton et al. 2008; Högberg, 2012; Schulte-Uebbing and De Vries, 2018; Du and De Vries 2018; Schwede et al. 2018). However, when the levels of N deposition are high (e.g., above 1.5–2.5 g N m<sup>-2</sup> year<sup>-1</sup>, De Vries et al. 2014a), the stimulating effect on forest growth is likely to diminish over time due to the accompanying side effects, such as soil acidification and imbalances between N and other nutrients such as phosphorous (P), calcium (Ca), and magnesium (Mg) (Aber et al. 1998; Bowman et al. 2008; De Vries et al. 2014b; Du and Fang 2014; Schwede et al. 2018).

The wet deposition in the Iranian Hyrcanian forests is about 3–5 g N m<sup>-2</sup> year<sup>-1</sup> (Salahi et al. 2014), which is considered as the critical amount (De Vries et al. 2014a). Despite the projection that the amount of N deposition in the Hyrcanian forests will be twofold (6–10 g N m<sup>-2</sup> year<sup>-1</sup>) by the end of this century (Galloway et al. 2008; Salahi et al. 2014; Nobakht et al. 2018), the effect of N deposition on the CO<sub>2</sub> efflux and fine root biomass and their consequences in this ecosystem are still unclear. As a common act to plant pioneer trees such as *Acer velutinum* Bioss. near the industrial areas after clearcutting, the main objective of this study was to quantify soil CO<sub>2</sub> efflux and fine root biomass in the different treatments of N addition to a maple (*Acer velutinum* Bioss.) plantation for the first time in Iran. It is hypothesized that the different rates of N addition (5, 10, 15 g N m<sup>-2</sup> year<sup>-1</sup>) significantly increase CO<sub>2</sub> efflux and fine root biomass in the maple plantation.

## 2 Materials and methods

### 2.1 Study area

This study was carried out in a maple (*Acer velutinum* Bioss.) plantation in the educational and research forest of Darabkola, located in the Western Hyrcanian forests, Sari City, Mazandaran Province, Iran (53° 16' East, 36° 31' North and an elevation of 360 m asl). The maple plantation was established following the clearing of a natural forest in 1990 (this stand is far from the industrial areas and highways in order to avoid the effect of those kinds of anthropogenic activities). The plantation area is 1.1 ha, and the average tree diameter (at breast height) and stand height is 20 ± 0.5 cm and 19 ± 1 m, respectively. The average slope and main aspects are 20% and north-west, respectively. The mean annual rainfall (1992–2015) is 753 mm. November is the rainiest month (114 mm), and June is the driest one (26 mm). The mean annual temperature is 18 °C (Anonymous 1996).

## 2.2 Experimental design

In April 2015, 12 rectangular 20- × 10-m plots were placed in a complete randomized design and each plot was surrounded by a 10-m-wide buffer strip (Appendix Fig. 5). Four treatments of N addition with three replicates (i.e., 3 plots for each treatment) were selected: 5 g N m<sup>-2</sup> year<sup>-1</sup> (N1), 10 g N m<sup>-2</sup> year<sup>-1</sup> (N2), 15 g N m<sup>-2</sup> year<sup>-1</sup> (N3), and control (without N addition, N0) (Xian-Kai et al. 2009). Nitrogen addition was conducted using ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) (Xu et al. 2007; Zhang and Han 2012).

N was added through spraying on the forest floor (litter layer) in this study. Starting from April 2015, the solution was sprayed monthly (on the third or fourth day after rainfall events) on the forest floor under the canopy with a backpack sprayer. The control plots received 20 L of distilled water without N addition (Tu et al. 2011). The study started 1 month after the first N addition and was carried out from May 2015 to April 2016.

## 2.3 Data collection

In April 2015, before the first N addition, three soil samples were taken from the topsoil layer (0–10 cm) in each plot (Xian-Kai et al. 2009; Wei et al. 2014; Liu et al. 2015), in a diagonal direction, using the coring method (diameter 8 cm; height 10 cm). Air-drying and grinding were performed, and the samples were passed through a 0.5-mm sieve. Then the physical and chemical properties of the soil were analyzed in the laboratory. Soil texture (hydrometer method, Bouyoucos 1951), bulk density (volumetric core method), pH (digital pH meter, in a 1:2.5 soil/water suspension), EC (EC meter, in water-saturated soil extract at 20 °C; Anonymous 1980), total N (Kjeltec System-Instrument, TECATOR; Anonymous 1990; Vogt et al. 2015), NO<sub>3</sub><sup>-</sup> (Manual Cd reduction method, APHA 1998), NH<sub>4</sub><sup>+</sup> (manual indophenols colorimetric method; Dorich and Nelson 1983), and organic carbon (Walkley and Black procedure) were measured.

Soil CO<sub>2</sub> efflux was measured using dynamic closed chambers. In April 2015, three polyvinyl chloride (PVC) collars (10 cm in diameter and 20 cm in height) were inserted into the soil at a depth of 10 cm (into organic and mineral soil), away from the edge of the plots, in order to measure soil CO<sub>2</sub> efflux (three points in each rectangular plot in a diagonal direction). Soil CO<sub>2</sub> efflux was measured once a month before each N addition by placing a PVC lid over each column and using the infrared method with a CO<sub>2</sub> port device (Messwert company GmbH-Göttingen), which was a developed version of the infrared gas analyzer, Edinburgh Sensors- Gascard II (Bekku et al. 1995; Hojjati 2008; Hojjati and Lamersdorf 2010). The measurement was done immediately after closing the chamber. The soil CO<sub>2</sub> efflux was measured three or four days after the rainfall events in order to avoid any pulse effect of precipitation. All the measurements were performed between 08:00 a.m. and 11:00 a.m. (local time).

Soil temperature (°C) and moisture were determined simultaneously with the soil CO<sub>2</sub> efflux measurements close to the soil collar in each plot (at three points in each rectangular plot in a diagonal direction). Soil temperature was measured using a digital thermometer (Model: 6300, Spectrum Technologies, Inc., USA) at the depth of 10 cm, and volumetric soil moisture was determined using a soil moisture meter (Model: DSM500, General Tools and Instruments, New York, USA) at the depth of 10 cm.

Fine root biomass was measured seasonally (once in the mid-season). In each plot, three soil cores (diameter: 8 cm; height: 10 cm) were taken at the depth of 10 cm (Argiroff et al., 2019; Li et al., 2021). Fine roots were collected after washing with a 2-mm sieve. To measure dry weight, all the samples were dried in an oven at 85 °C (Zhang et al. 2014).

## 2.4 Statistical analysis

The effect of N addition treatments and month on soil temperature, moisture, CO<sub>2</sub> efflux, and fine root biomass were tested by Repeated measures analysis of variance for

**Table 1** Physical and chemical properties (mean values ± SE, n = 3) of soil (depth of 0–10 cm) in each treatment in the *Acer velutinum* Bioss. stand before N addition (April 2015)

Soil properties	Treatment plots			
	N0	N1	N2	N3
% Sand:Silt:Clay	58:30:12	50:34:16	60:28:12	56:30:14
Bulk density (g cm <sup>-3</sup> )	1.72 ± 0.02	1.77 ± 0.02	1.69 ± 0.01	1.70 ± 0.02
pH	7.17 ± 0.01	7.20 ± 0.01	7.18 ± 0.01	7.12 ± 0.02
Electrical conductivity (dS m <sup>-1</sup> )	0.89 ± 0.02	0.91 ± 0.01	0.90 ± 0.09	0.87 ± 0.03
Total N (g 100 g <sup>-1</sup> )	0.38 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.39 ± 0.01
NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	4.13 ± 0.13	4.08 ± 0.09	4.15 ± 0.07	4.19 ± 0.10
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	4.87 ± 0.05	4.79 ± 0.07	4.71 ± 0.08	4.85 ± 0.10
OC (g 100 g <sup>-1</sup> soil)	3.94 ± 0.04	4.08 ± 0.18	3.85 ± 0.16	3.95 ± 0.06
Fine root biomass (g m <sup>-2</sup> )	46.9 ± 0.95	47.1 ± 3.59	49.8 ± 0.73	46.8 ± 1.95

**Table 2** Total chemical properties (mean values  $\pm$ SE,  $n = 3$ ) of soil (depth of 0–10 cm) in each treatment in the *Acer velutinum* Bioss. plantation after N addition at the end of study period (April 2016). Different letters represent significant differences between treatments within each property

Soil properties	Treatment plots			
	N0	N1	N2	N3
pH	7.13 $\pm$ 0.01 a	7.08 $\pm$ 0.01 b	6.86 $\pm$ 0.01 c	6.76 $\pm$ 0.01 d
Electrical conductivity (dS m <sup>-1</sup> )	0.84 $\pm$ 0.03 a	0.78 $\pm$ 0.01 b	0.75 $\pm$ 0.01 c	0.66 $\pm$ 0.01 d
Total N (g 100 g <sup>-1</sup> )	0.36 $\pm$ 0.01 d	0.41 $\pm$ 0.01 c	0.47 $\pm$ 0.02 b	0.58 $\pm$ 0.01 a
NH <sub>4</sub> <sup>+</sup> (mg kg <sup>-1</sup> )	4.93 $\pm$ 0.18 d	6.04 $\pm$ 0.14 c	7.23 $\pm$ 0.10 b	8.53 $\pm$ 0.17 a
NO <sub>3</sub> <sup>-</sup> (mg kg <sup>-1</sup> )	5.06 $\pm$ 0.18 d	7.21 $\pm$ 0.12 c	9.95 $\pm$ 0.30 b	20.5 $\pm$ 0.33 a

the study period (significance was at 0.05 level). One-way ANOVA was used to test the difference in soil chemical properties in each treatment after N addition at the end of study period. Mean values in the text are averages of 3 plots per N addition treatment (N0, N1, N2, and N3). The relationships between soil CO<sub>2</sub> efflux and soil temperature were determined using nonlinear regression models (exponential equation,  $R = ae^{bT}$ ). For the regression models, the standard error of estimation (Std. Err. Est) and the normalized root-mean-square error (RMSE) were calculated. All the statistical analyses were performed using the IBM SPSS statistic 26 software (USA). Excel software was used to draw statistical graphics.

### 3 Results

#### 3.1 Soil properties

The results showed no significant difference in the soil properties between the treatments before the N addition (Table 1). At the end of the study period, N addition (N2 and N3) led to lower soil pH and EC. Moreover, after the

completion of the study period, the soil total N, ammonium (NH<sub>4</sub><sup>+</sup>), and nitrate (NO<sub>3</sub><sup>-</sup>) were significantly higher in the N addition plots than the control plots and they also significantly increased from N0 to N3 (Table 2).

There was no significant difference ( $P > 0.05$ ) between each N addition treatments and the control for each month (Table 3). Soil temperature and moisture exhibited distinct seasonal patterns in all the treated plots. The highest and lowest soil temperatures were observed in July and January, respectively (Fig. 1a). The highest and lowest soil moistures were observed in January and July, respectively (Fig. 1b).

#### 3.2 Effects of N addition on soil CO<sub>2</sub> efflux

ANOVA results revealed that N additions had a significant effect ( $P < 0.01$ ) on soil CO<sub>2</sub> efflux (Table 3). The highest N addition resulted in a higher rate of CO<sub>2</sub> efflux than the other treatments during the study period (Fig. 2a). In all plots, mean soil CO<sub>2</sub> efflux was significantly higher during the growing season (spring and summer) than winter. The annual averages of CO<sub>2</sub> effluxes were  $115 \pm 3.00$ ,  $122 \pm 7.3$ ,

**Table 3** ANOVA results on soil temperature, moisture, CO<sub>2</sub> efflux, and fine root biomass, based on repeated measurements (months, N addition, and their interaction during the study period)

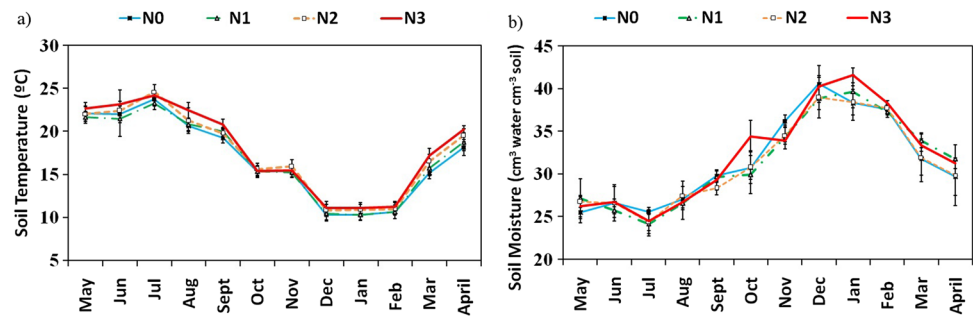
Soil properties	Source	df <sup>a</sup>	Mean square	F
Soil temperature	Month	11	276.9	1044.009**
	Month $\times$ Nitrogen addition	33	0.534	2.014**
	Nitrogen addition	3	8.194	0.272 <sup>ns</sup>
Soil moisture	Month	11	333.387	154.666**
	Month $\times$ Nitrogen addition	33	2.937	1.362 <sup>ns</sup>
	Nitrogen addition	3	2.172	0.182 <sup>ns</sup>
Soil CO <sub>2</sub> efflux	Month	11	21920.442	648.249**
	Month $\times$ Nitrogen addition	33	698.675	20.662**
	Nitrogen addition	3	24097.282	429.214**
Fine root biomass	Season	3	11.942	13.674**
	Season $\times$ Nitrogen addition	9	0.694	0.794 <sup>ns</sup>
	Nitrogen addition	3	42.360	18.494**

*ns* not significant

\*\*Significance at the level of 0.01

<sup>a</sup>Degree of freedom

**Fig. 1** **a** Seasonal variations (mean  $\pm$  SD;  $n = 3$  per month) of the soil temperature; **b** seasonal variations (mean  $\pm$  SD;  $n = 3$  per month) of the soil moisture measured at control plot (no N addition) at depth of 10 cm

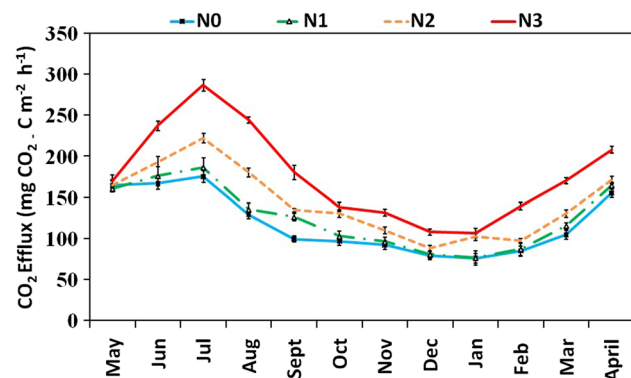


$141 \pm 10.7$ , and  $174 \pm 16.1$  mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in N0, N1, N2, and N3, respectively.

Soil CO<sub>2</sub> efflux followed a seasonal pattern with maximum and minimum during summer and winter, respectively (Fig. 2). The rates for CO<sub>2</sub> efflux in the control plots ranged from  $76.0 \pm 1.7$  mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in July to  $175 \pm 6.1$  mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in January. There was no significant difference between N0 and N1; however, a significant difference was observed between N0, N2, and N3. Soil CO<sub>2</sub> efflux was exponentially related to the soil temperature (Fig. 3). Exponential relationships between the soil CO<sub>2</sub> efflux and soil temperature were significant ( $P < 0.01$ ) for all the treatments with  $R^2$  values between 0.81 and 0.87.

### 3.3 Effects of N addition on root biomass

The results indicated an effect of N addition ( $P < 0.01$ ) on fine root biomass (Table 3). There was no difference between N0, N1, and N2; however, fine root biomass was significantly higher in N3 than the other treatments (Table 4). The relationship between soil CO<sub>2</sub> efflux and fine root biomass was exponential (Fig. 4). Exponential relationships between fine root biomass and soil CO<sub>2</sub> efflux were significant ( $P < 0.01$ ) for all the treatments with  $R^2$  values between 0.41 and 0.59.



**Fig. 2** Seasonal variations (mean  $\pm$  SD;  $n = 3$  per month) of soil CO<sub>2</sub> efflux measured at different level of N addition (N0: Control, N1: 5, N2: 10, and N3: 15 g N m<sup>-2</sup> year<sup>-1</sup>)

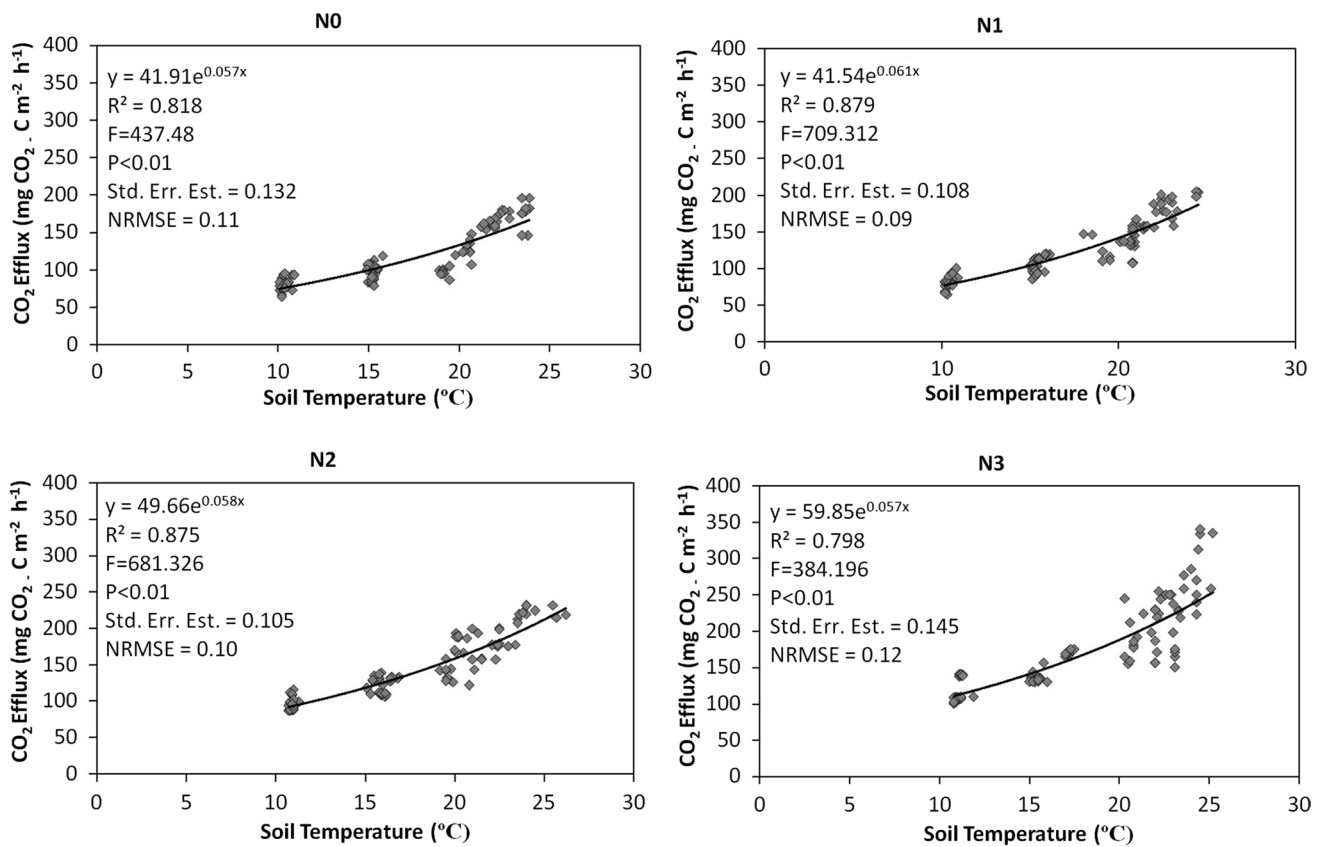
## 4 Discussion

According to the results, N additions increased the available N concentration at the end of the study period. Nitrogen addition directly affects the soil N cycling by enhancing the available N and also has an indirect effect on the rate of nutrient input by altering the litter decomposition and nutrient release. Geng et al. (2017) and Zhang et al. (2019) reported that the N addition significantly increased the available N in the soil. In the N-limited forest ecosystems such as Hyrcanian forests, adding N to the soil can increase the net N mineralization in the forest floors, which can increase the available concentration of N in the soil (Gundersen et al. 1998; Zhu et al. 2015a, b; Gao et al. 2015; Ye et al. 2018).

In the current study, N additions significantly decreased soil pH and EC. Reduction in soil pH following N additions has been reported in other studies as well (Hong et al. 2019; Guo et al. 2010; Yang et al. 2012; Yang et al. 2015). Nitrogen addition is known as the main cause of the reduction of soil pH (Guo et al. 2010; Yang et al. 2012, 2015). Adding N to the soil, especially as NO<sub>3</sub><sup>-</sup>, may enhance the co-leaching loss of the base cations (e.g., K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>) (Gundersen et al. 2006; Lu et al. 2018).

The present results showed that N2 and N3 (10 and 15 g N m<sup>-2</sup> year<sup>-1</sup>) significantly increased annual soil CO<sub>2</sub> efflux. Increased soil CO<sub>2</sub> efflux by N addition has also been reported in previous studies (Tu et al. 2013; Gao et al. 2014; Deng et al. 2018; Li et al. 2019; Zhao et al. 2020). There was no significant difference between the different treatments in the terms of soil temperature, moisture, or physical and chemical properties before the N addition. Therefore, it can be stated that the difference in the CO<sub>2</sub> efflux in the different treatments might have been caused by the N addition. Soil CO<sub>2</sub> efflux includes heterotrophic and autotrophic respiration and is mainly regulated by the size of the soil microbial population, amount of fine roots, and biomass of litter (Baggs 2006). It has been reported that adding N to N-limited forests, such as the Hyrcanian forest soils in this study, can increase the soil CO<sub>2</sub> efflux rate (Li et al. 2019; Zhang et al. 2017b). Moderate addition of N to N-limited forests





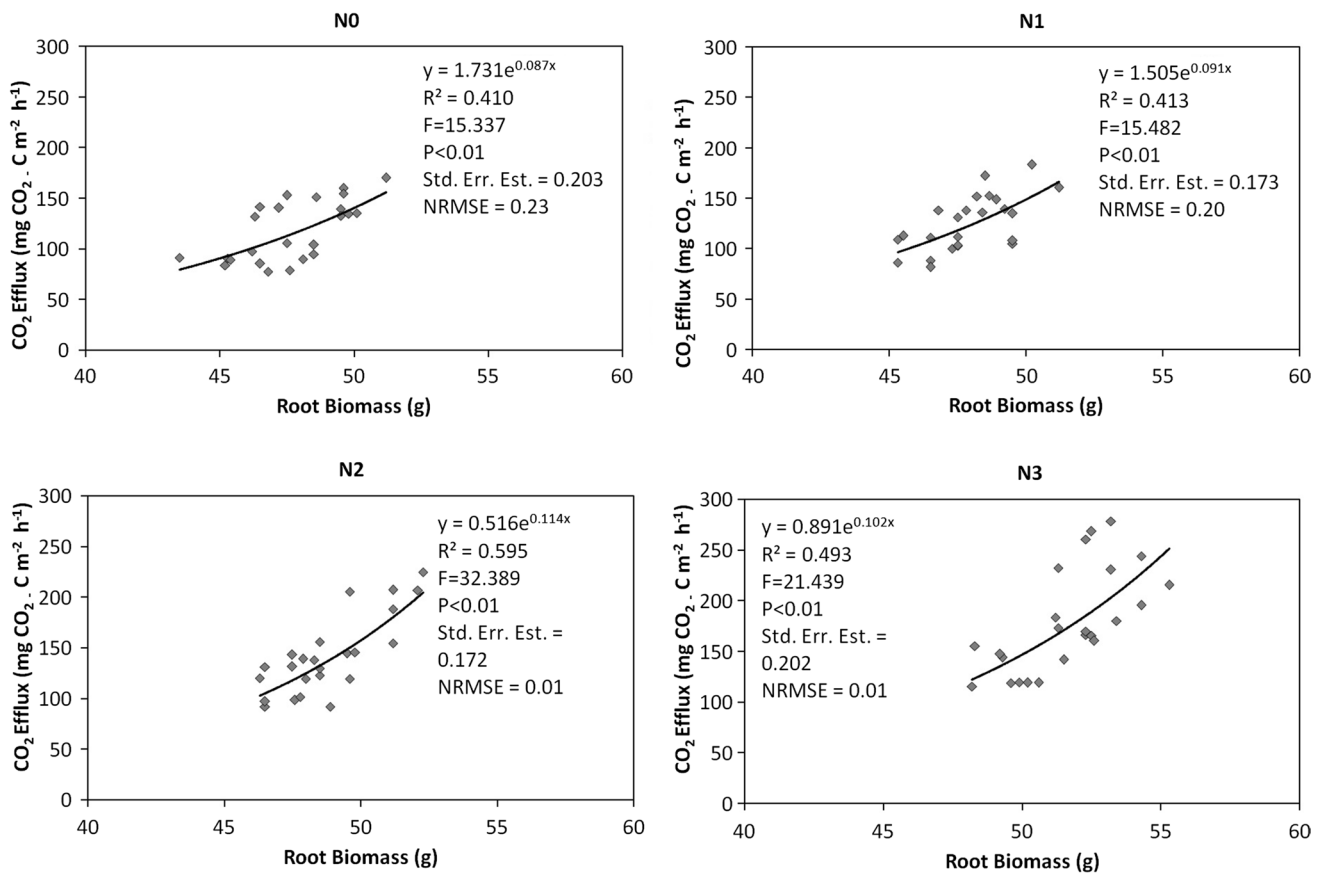
**Fig. 3** Relationships between soil CO<sub>2</sub> efflux and soil temperature measured at different levels of N addition (N0: Control, N1: 5, N2: 10, and N3: 15 g N m<sup>-2</sup> year<sup>-1</sup>); Std. Err. Est standard error of estimate, NRSME normalized RMSE

may increase the N content of soil and thus decrease the soil C/N, which is beneficial to the microbial biomass and activity as well as the plant growth (Chu et al. 2010; Li et al. 2016) and therefore enhances soil CO<sub>2</sub> efflux. Moreover, the addition of N to the soil increases the decomposition rates of leaf litter and fine root, which provides more substrates for the microbial communities and finally increases the microbial biomass (Li et al. 2016), enzyme activity, and microbial respiration. On the other hand, C input from the additional aboveground litter and decomposed roots eventually enters into the soil organic

**Table 4** The fine root biomass (g m<sup>2</sup>) (mean values ± SE) in different treatments. Different letters represent significant differences between treatments within each season (the starting point of this study was summer and finished at the end of spring)

	Summer	Fall	Winter	Spring
N0	48.6 ± 1.21 b	46.6 ± 0.60 b	47.2 ± 0.31 b	48.2 ± 0.67 b
N1	49.0 ± 0.65 b	47.3 ± 0.19 b	47.0 ± 0.59 b	48.2 ± 0.41 b
N2	49.9 ± 0.32 b	47.8 ± 0.43 b	47.6 ± 0.53 b	49.9 ± 0.56 b
N3	52.8 ± 0.61 a	51.3 ± 1.21 a	50.1 ± 0.45 a	52.2 ± 0.44 a

matter and stimulates heterotrophic respiration (Bowden et al. 2004; Phillips et al. 2007). Burton et al. (2004) also claimed that NO<sub>3</sub><sup>-</sup> addition increased the soil CO<sub>2</sub> efflux in a sugar maple stand in Michigan during the first year of the investigation. A similar response to N additions (5 and 15 g N m<sup>-2</sup> year<sup>-1</sup>) has been observed in an oak (*Quercus velutina*) dominated hardwood in Harvard Forest, with increased soil CO<sub>2</sub> efflux occurring in the first year of N addition (Bowden et al. 2004). Mo et al. (2008) in their study in an old-growth broadleaf forest (monsoon evergreen) observed that N deposition (50 kg N ha<sup>-1</sup> year<sup>-1</sup>) enhanced soil CO<sub>2</sub> efflux and high N addition rates (≥ 100 kg N ha<sup>-1</sup> year<sup>-1</sup>) reduced it, whereas N1 (50 kg N ha<sup>-1</sup> year<sup>-1</sup>) had no significant effect and N2 (100 kg N ha<sup>-1</sup> year<sup>-1</sup>) increased the soil CO<sub>2</sub> efflux in the present study. Some studies have shown the declining or insignificant effects of N deposition on soil CO<sub>2</sub> efflux (Burton et al. 2004; Jiang et al. 2010; Wei et al. 2014; Samuelson et al. 2009; Krause et al. 2013). These inconsistent results might be related to the N addition rates, initial N condition of the soil, soil properties, and the tree species (Gao et al. 2015; Li et al. 2019).



**Fig. 4** Relationships between soil CO<sub>2</sub> efflux and fine root biomass measured at different N addition (N0: Control, N1: 5, N2: 10, and N3: 15 g N m<sup>-2</sup> year<sup>-1</sup>); Std. Err. Est Standard Error of Estimate, NRSME normalized RMSE

The results of the current study confirmed that soil CO<sub>2</sub> efflux was exponentially related to soil temperature. This is in line with findings in the temperate forests (Bowden et al. 2004; Samuelson et al. 2009) and the subtropical/tropical moist ones (Mo et al. 2008). Moreover, noticeable exponential and linear relationships between soil CO<sub>2</sub> efflux and soil temperature have been reported for other vegetation types (Fang and Moncrieff 2001; Samuelson et al. 2004; Jassal et al. 2007; Wang et al. 2019). In the present study, the soil CO<sub>2</sub> efflux in the maple plantation stand exhibited a strong seasonal pattern, which reached the maximum rate in the midsummer and the minimum rate in the late winter. Distinct seasonal patterns of the soil efflux relevant to the soil temperature and moisture and plant growth have also been found in other studies (Contosta et al. 2011; Du et al. 2011; Wang et al. 2019).

The treatment of N3 (15 g N m<sup>-2</sup> year<sup>-1</sup>) significantly increased fine root biomass. Increased fine root biomass by N addition (8–12 g N m<sup>-2</sup> year<sup>-1</sup>) has also been reported in various studies (Cleveland and Townsend 2006; Xu and Wan 2008; Song et al. 2017; Li et al. 2019; Wang et al.

2019; Ren et al. 2019). According to the minimum limiting factors theory, the increase of N in the soil may intensify the deficiency of other nutrients in the soil. Fine roots need to increase root growth to uptake more nutrients (Wang et al. 2013; Zhang et al. 2020). Yan (2017) reported that the deposition of N to soil may decrease the fine root surface area, but can increase the thickness of fine roots and subsequently may result in an increase in the total biomass of the fine roots. Other studies have found that trees at different growth stages exhibit different characteristics due to their ecological plasticity; fine root production and turnover rates of young trees usually increased with the increase of soil N availability (Børja et al. 2008; Jagodzinski and Katuckd 2011; Xiong et al. 2018).

## 5 Conclusions

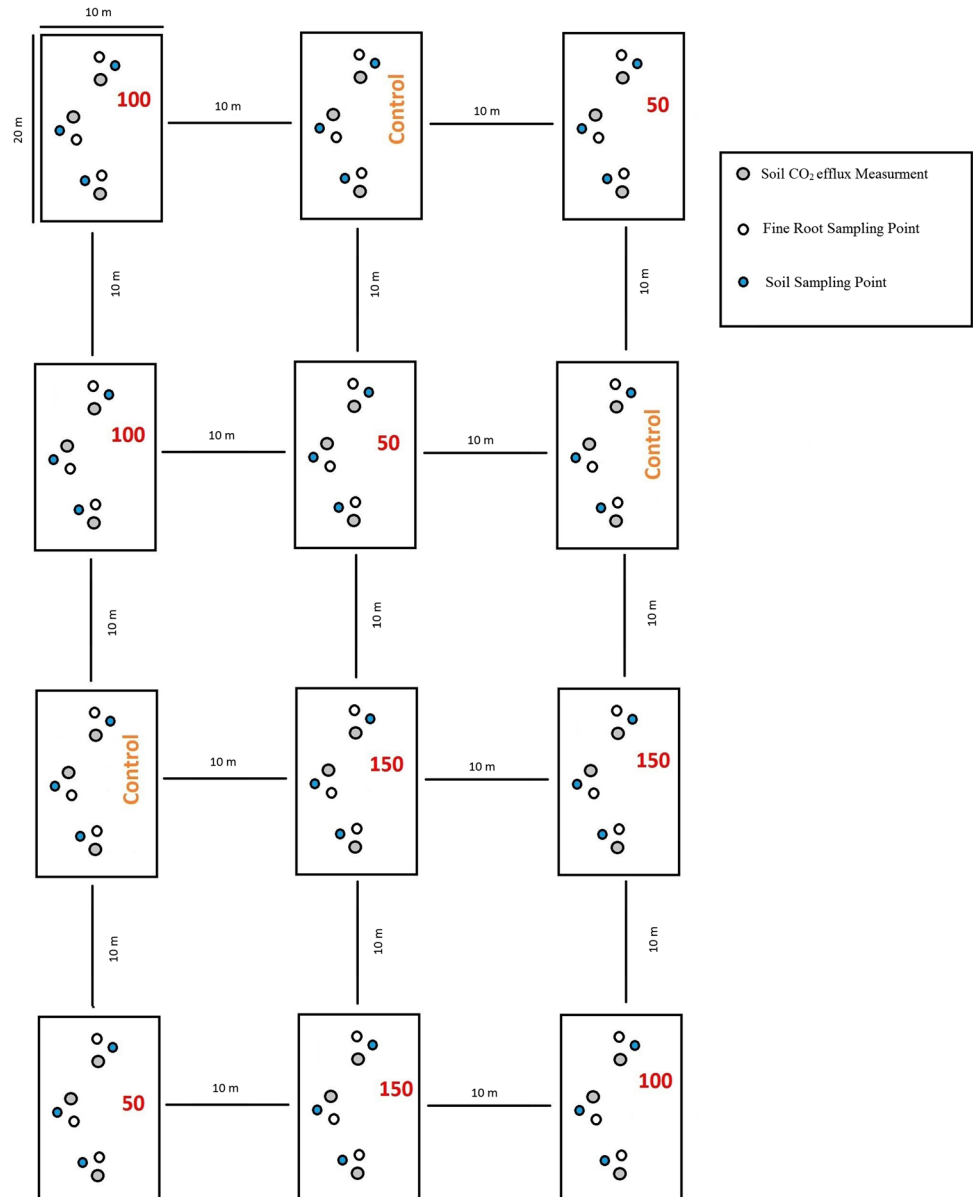
The current study presented new data on the soil CO<sub>2</sub> efflux in the Hyrcanian forest. According to the results, increased N availability (10 and 15 g N m<sup>-2</sup> year<sup>-1</sup>) and increased temperature stimulated the fine root biomass, which was

observed along with an increase in the soil CO<sub>2</sub> efflux in the maple (*Acer velutinum* Bioss.) plantation stand. N addition (10 and 15 g N m<sup>-2</sup> year<sup>-1</sup>) led to a lower soil pH and EC, and all N addition treatments (5, 10, and 15 g N m<sup>-2</sup> year<sup>-1</sup>) led to an increase in soil total N, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> at the end of the study period. It is projected that the amount of N deposition in the Hyrcanian forests might be doubled by the end of this century (6–10 g N m<sup>-2</sup> year<sup>-1</sup>); therefore, soil CO<sub>2</sub> efflux due to N deposition could be an important challenge in the future. Since the effect of N addition on forest stands is

a long-term and complex process, further long-time studies would be needed to clarify the response of the root respiration and enzyme activity to increased N availability. Such studies in these valuable forests could lead to a comprehensive understanding of the effects of the increased N amount on the C cycle.

## Appendix

**Fig. 5** Layout of the experimental design (N1: 5, N2: 10, and N3: 15 g N m<sup>-2</sup> year<sup>-1</sup>)





**Acknowledgements** The authors gratefully acknowledge University of Göttingen, Institute of Soil Science, and Dr. Mohsen Zarebanadkouki, for their kind cooperation.

**Funding** This study was funded by Sari Agricultural Sciences and Natural Resources University.

**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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