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ORIGINAL PAPER

Earthworm population and microbial activity temporal dynamics in a Caspian Hyrcanian mixed forest

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Abstract Few studies have analyzed how tree species within a mixed natural forest affect the dynamics of soil chemical properties and soil biological activity. This study examines seasonal changes in earthworm populations and microbial respiration under several forest species (*Carpinus betulus*, *Ulmus minor*, *Pterocarya fraxinifolia*, *Alnus glutinosa*, *Populus caspica* and *Quercus castaneifolia*) in a temperate mixed forest situated in northern Iran. Soil samplings were taken under six individual tree species ($n = 5$) in April, June, August and October (a total of 30 trees each month) to examine seasonal variability in soil chemical properties and soil biological activity. Earthworm density/biomass varied seasonally but not significantly between tree species. Maximum values were found in spring (10.04 m^{-2}/16.06 mg m^{-2}) and autumn (9.7 m^{-2}/16.98 mg m^{-2}) and minimum in the summer (0.43 m^{-2}/1.26 mg m^{-2}). Soil microbial respiration did not differ between tree species and showed similar temporal trends in all soils under different tree species. In contrast to earthworm activity, maximum microbial activity was measured in summer (0.44 mg CO_2–C g $soil^{-1}$ day^{-1}) and minimum in winter (0.24 mg CO_2–C g $soil^{-1}$ day^{-1}). This study shows that although tree species affected soil chemical properties (pH, organic C, total N content of mineral soils), earthworm density/biomass and microbial respiration are not affected by tree species but are controlled by tree activity and climate with strong seasonal dynamics in this temperate forest.

Keywords Mixed forest · Deciduous trees · Macrofauna activity · Microbial respiration

Introduction

The Hyrcanian vegetation zone, also called Caspian forest, is a green belt stretching over the northern slopes of the Alborz mountain ranges and covers the southern coast of the Caspian Sea (Talebi et al. 2014). These mixed forests are one of the last remnants of natural deciduous forests in the world (Kooch et al. 2014). While the Caspian coastal areas enjoy a milder climate, the inland plateau experiences extremes of temperature in summer and winter (Talebi et al. 2014). These mixed forests are one of the most diverse in the area and are composed of several species. The overstory composition of a forest affects soil biological and physical–chemical functioning (Augusto et al. 2015). Understanding how tree species affect soils is important because soils are a fundamental component of ecosystems, and changes in soil properties are likely to change future vegetation development at a given site (Frouz et al. 2013). In order to obtain a broader insight into how single forest species affect soils, aspects such as biological activity and soil physicochemical properties should be taken into consideration (Neirynck et al. 2000). Tree species affect physical, chemical and biological characteristics of soils in several ways depending

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on leaf and root traits (Wang et al. 2009). Additionally, spatial and temporal variability are crucial to understand soil functionality in forest ecosystems. Up to now, most studies have examined physical and chemical properties of soils, but its biological activity is rarely considered (Frouz et al. 2013). The effect of trees on soil physical and chemical properties can be mediated by soil fauna, which may substantially affect topsoil properties as a consequence of bioturbation (Frouz et al. 2013). Recent studies recognize soil fauna as one of the important factors for evaluating soil quality and health (Holdsworth et al. 2012; Frouz et al. 2013). Among these, macrofauna plays a crucial role in food and energy cycles, affecting mainly soil organic matter dynamics (Barrios 2007). Among soil macrofauna, earthworms account for the greatest invertebrate biomass (Tondoh et al. 2007; Blouin et al. 2013). Previous studies (Suthar 2012; Sackett et al. 2013; Crumsey et al. 2014) have described the main factors influencing earthworm activity, including tree species, soil and litter characteristics (such as soil pH and litter palatability) and environmental factors (such as soil temperature and moisture). Meanwhile, earthworms exert a key role in nutrient cycling through their own metabolism increasing carbon and nitrogen, stimulation of microbial activity and bioturbation or physical mixing of organic and mineral soil (Suthar 2012).

The presence of different tree species having different above- and belowground functional traits may result in distinct soil microbial communities with different abilities to resist and recover after a disturbance (Rivest et al. 2015). Soil respiration is a major process controlling carbon (C) loss from terrestrial ecosystems (Huang et al. 2014; Makita and Fujii 2015; Rey 2015) and a good indicator of total belowground C allocation and ecosystem productivity (Davidson et al. 2000). How tree species impact soil microbial respiration is still limited, but is crucial to guide selection of species for reforestation and carbon management purposes (Makita and Fujii 2015). The activity of soil biota is strongly driven by environmental factors such as soil temperature, soil moisture, bulk density and pH. Moreover, soil community composition regulates soil carbon and nitrogen dynamics (Huang et al. 2014; Sherman et al. 2015). Soil temperature and soil water content are the main environmental factors controlling the temporal variation in CO_2 emission from soils (Davidson et al. 2000; Rey et al. 2002). However, the direct effect of tree species on both soil autotrophic and heterotrophic respiration is less well understood, because of the strong interaction between abiotic (e.g., soil moisture, temperature and texture) and biotic (e.g., quality and quantity of organic matter input, soil functioning) factors (Huang et al. 2014). In temperate forest ecosystems, soil temperature is the most important factor controlling the spatial and temporal variation of soil heterotrophic respiration, while soil moisture is secondary, only important under drought conditions (Huang et al. 2014). Tree species can influence the composition and functioning of the underlying soil microbial communities (Garbeva et al. 2006) through differences in tree canopy cover, litter quality and quantity (Gruselle and Bauhus 2010). On the other hand, many studies have revealed strong correlations between microbial community structure and an array of environmental factors, including pH, quantity and quality of C and N (e.g., Cleveland et al. 2014). The variability of environmental conditions, temperature and moisture regimes can strongly affect soil biological activity (Qiu-Hui et al. 2012) and biochemical properties (Guicharnaud et al. 2010; Zhu and Cheng 2013), nutrient cycling (Mikha et al. 2005) and site productivity (Sajedi 2010). In order to gain better understanding on the spatial and temporal distribution of earthworm populations and microbial activity, we carried out a study in a mixed forest located in Northern Iran. The aim of this study was to investigate: (1) how tree species affect soil physicochemical properties and, in turn, earthworm density/biomass and microbial activity, (2) seasonal changes in earthworm and microbial activity under different forest species within the forest and (3) which factors determine earthworm and microbial activity in these temperate forests.

Materials and methods

Site characteristics

The study area is located at the experimental forest station of Tarbiat Modares University, north of Iran (51°46”E, 36°47″N) (Fig. 1a, b). The experimental plots were located at an altitude of 15 m above sea level. The area is flat and uniform (slope 0–3%). Mean annual temperature is 17 °C, and mean annual rainfall is 803.4 mm with a dry season between May and August (Fig. 2). The parent material is dolomite limestone that belongs to upper Jurassic and lower Cretaceous periods. The soil is of the order Alfisols, with a silty clay loam texture (Kooch et al. 2012). The natural forest vegetation is temperate deciduous forests containing broadleaved species dominated by oak (*Quercus castaneifolia* C. A. M. *macranthera* F. and M.), hornbeam (*Carpinus betulus* L.), elm (*Ulmus minor* Mill., *Ulmus glabra* Huds.), Caucasian wingnut (*P. fraxinifolia*), Persian poplar (*Populus caspica* Bornm.) with some associated species such as maple (*Acer velutinum* Boiss., *Acer cappadocium* Gled.), ash (*Fraxinus excelsior* L.), alder (*Alnus subcordata* C. A. M., *Alnus glutinosa* Gaertn.), wild cherry (*Prunus avium* L.), wild service tree (*Sorbus torminalis* Crantz) and lime tree (*Tilia platyphyllus* Scop.) (Mirzaei et al. 2007). According to a previous report (Anonymous

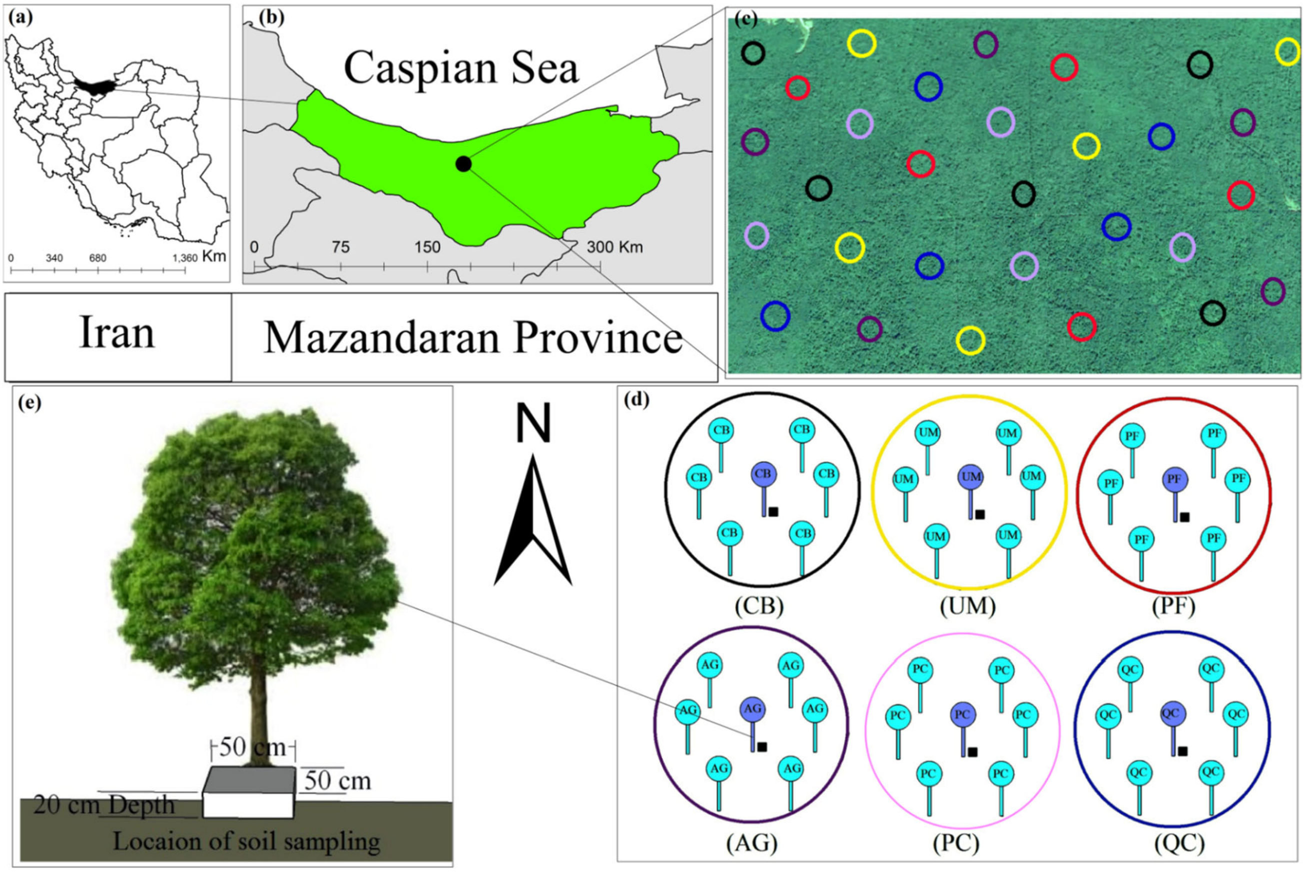


Fig. 1 Location of the study site in the Hyrcanian zone, the Central Caspian region of northern Iran (**a**, **b**). Study site with 50 ha area including clumps of tree species [*C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC)] (**c**, **d**). Five replications for each tree species with same diameter class of ≈65 cm were considered ($n = 30$); soil samples with 0.5 m^2 to a 20 cm depth (**e**) were taken under tree canopy cover in April, June, August and October (N total = 120). These species were surrounded by same tree species (the schematic design not in scale)

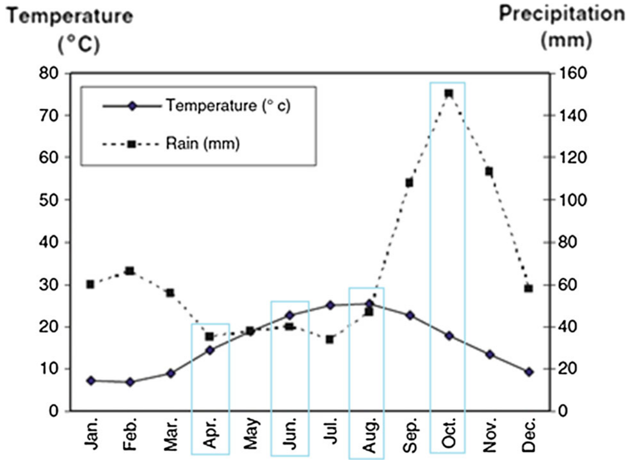


Fig. 2 Mean monthly air temperature (°C) and precipitation (mm) at the study site (Meteorological station at the Noushahr city)

2015), the variations in soil physical characteristics [i.e., soil bulk density (1.35–1.56 g cm^{-3}) and texture (20.40–25.20% for sand; 38.60–48.20% for silt and 29.20–36.40% for clay)] were not significantly different among tree species in the study area. However, litter thickness and C/N ratio were significantly higher under oak (15.72 cm and 61.16, respectively) and the least were found under alder (6.29 cm and 21.35, respectively) species.

Soil sampling and analyses

In this study, the effect of individual tree species on soil chemical and biological features in mixed Caspian plain temperate forests with area of 50 hectares was investigated. For all species, weather conditions, topography (elevation and slope), geology and soil types were identical. All tree species were identified in the study area. Samplings were performed under six species of C. *betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC). Five individuals of each tree species (with same diameter class of ≈65 cm) were considered (in total 30 trees each month), and soil samples underneath each tree ($n = 120$) were taken in April, June, August and October. The selected trees were surrounded by individuals of the same tree species (Fig. 1c, d). Earthworms were collected by a combination of hand sorting and mustard extraction (on the whole sampling area of 0.5 m^2 to a 20 cm depth; Fig. 1e), followed by hand sorting of a soil sample (Hlava and Kopecký 2013). Sampling of earthworms was conducted in the soil volume after the removal of litter (Scullion et al. 2014). Then, whole of soils related to one sample were completely mixed in the field (0.5 m^2 to a 20 cm depth), and just some parts ($\approx$500 g) were taken to the laboratory. Soil samples for microbial respiration determination were immediately transferred to a cooled, insulated container for transport to the laboratory and were stored at 4 °C until they were processed. Soils for chemical analyses were air-dried and passed through a 2-mm sieve (aggregates were broken). We determined the following soil properties: soil water content by drying soil samples at 105 °C for 24 h, soil pH using an Orion Analyzer Model 901 pH meter in a 1:2.5, soil/water solution, soil organic C using the Walkley–Black technique (Allison 1975) and total N using a semi-micro-Kjeldahl technique (Bremner and Mulvaney 1982). The collected earthworms were washed and weighed to milligram precision after oven drying at 60 °C for 24 h for earthworm biomass determination (Kooch et al. 2014). Species of earthworms were identified (epigeic, anecic and endogeic) by external characteristics using the key of Edwards and Bohlen (1996). Soil microbial respiration was determined by measuring the CO_2 evolved in a 3-day incubation experiment at 25 °C, in which 50 g of each soil sample (re-moistened to 55% its water holding capacity) was placed in a glass jar. A glass vial holding 10 mL of 0.5 M NaOH was placed in a glass jar to trap the evolved CO_2. The excess alkali, after precipitating the CO_3^{2-} with 0.5 M $BaCl_2$ solution, was titrated with standard 0.5 M HCl to a phenolphthalein end point (Alef 1995).

Statistical analysis

Three-way analysis of variance (ANOVA) with repeated measures on one factor (time) were used to compare soil properties between tree species over time. When factors were significant ($P < 0.05$), post hoc tests were done using a Duncan's multiple comparison test. All statistical analyses were conducted using the SPSS v. 20 statistical software package. Data were checked to meet the assumptions of normality by the Kolmogorov–Smirnov test and homogeneity of variances by the Levene's test. Relationships between earthworm density, biomass (earthworm communities) and chemical properties of soils and microbial activity under the six tree species across different seasons (months) were analyzed by principle component analysis (PCA) using PC-Ord version 5.0 (McCune and Mefford 1999).

Results

Soil water content and soil chemical properties

Overall, tree species affected soil chemical properties (Table 1). All soil properties showed statistically significant differences among different tree species: soil water content ($P = 0.006$), pH ($P = 0.001$), soil organic C content ($P < 0.001$), total N ($P < 0.001$) and C/N ratio ($P < 0.001$) (Table 1). Soil organic C was significantly higher in all months under QC when compared with the other tree species (Table 2). Total N was significantly higher in soils under AG, and soil C/N ratio was significantly higher under the QC when compared with the other studied tree species (Table 2). Except for C/N ratio ($P > 0.05$), all soil physicochemical properties varied with season (Table 1). Maximum soil organic C ($P = 0.003$) and total N ($P < 0.001$) were measured in August (Tables 1, 2). Water content ($P < 0.001$) and pH ($P < 0.001$) were significantly higher in April compared with values measured in the other months (Tables 1, 2).

Soil biological activity

Soil biological activity was not significantly different among tree species ($P > 0.05$), but changed seasonally in the same way in all tree species (Table 1). Earthworm density/biomass ($P < 0.001$), epigeic density/biomass ($P < 0.001$), anecic density/biomass ($P < 0.001$), endogeic density/biomass ($P < 0.001$) and microbial respiration ($P < 0.001$) varied significantly over time (Table 1). The amount of earthworm density/biomass was maximum in April and October and minimum in August (Figs. 3, 4). Similarly, soil microbial respiration was maximum in April and minimum in October (Fig. 5). From the PCA output, the first PC explained more than 60% of variance in soil properties under studied tree species in different seasons (Fig. 6). The right PC1 shows that under conditions with more water content, pH favored higher activity of earthworms (April and October), while the left PC1 shows that water content, pH and earthworms activity were minimum over the summer (June and August) under different studied tree species (Fig. 6). In the case of soil microbial respiration, the PC2 explained less than 20% of the variance, with soil water content, pH, organic C and total N explaining microbial activity in August (as significant under AG tree species) and April (Fig. 6).

Table 1 Three-way analysis of variance (ANOVA) with repeated measures soil water content, chemical and biological properties in relation to different tree species and months

Soil features	Tree species		Time		Tree species × time	
	F	*P*	*F*	*P*	*F*	*P*
Physical and chemical variables						
Water content	4.493	**0.006**	79.962	**0.000**	1.165	0.319
pH	5.770	**0.001**	22.000	**0.000**	72.00	**0.000**
C	27.637	**0.000**	5.146	**0.003**	0.261	0.997
N	12.560	**0.000**	13.203	**0.000**	0.793	0.618
C/N ratio	10.518	**0.000**	0.718	0.544	2.181	**0.015**
Biological variables						
Earthworm density	1.225	0.328	76.723	**0.000**	1.312	0.218
Earthworm biomass	1.431	0.249	87.166	**0.000**	1.703	0.069
Epigeic density	1.072	0.400	53.782	**0.000**	1.202	0.290
Epigeic biomass	1.259	0.314	57.994	**0.000**	1.589	0.099
Anecic density	0.623	0.684	17.926	**0.000**	0.644	0.829
Anecic biomass	0.561	0.729	22.159	**0.000**	0.697	0.779
Endogeic density	0.820	0.548	4.250	**0.008**	0.850	0.620
Endogeic biomass	0.757	0.589	4.358	**0.007**	0.787	0.687
Soil microbial respiration	0.045	0.390	9.946	**0.000**	1.788	0.053

Bold numbers indicate significant statistical differences

Discussion

Earthworm activity

Our main objective was to investigate whether tree species within a forest affect earthworm diversity and activity. Since forest tree species affect soil physicochemical properties, we expected changes in earthworm activity. In contrast to other studies that have found that earthworm population are affected by tree species (e.g., differences in litter quality) and/or soil features (e.g., pH, nutrient availability) (Kooch et al. 2012; Crumsey et al. 2014), our study did not show significant statistical differences in earthworm density/biomass among tree species. Instead, our results showed temporal changes in earthworm density/biomass driven by seasonal changes in environmental conditions. Indeed, environmental conditions play an important role in the functional categories of earthworms present in an ecosystem (Chaudhuri and Paliwal 2008; Suthar 2012) causing spatial and temporal heterogeneity in earthworm populations. Soil moisture and soil temperature are usually inversely correlated in this climatic zone and act synergistically to influence earthworm activity (Day and Chaudhur 2014). Indeed, maximum earthworm activity was recorded in April and October, when soil moisture and soil temperature are favorable, whereas in August, the driest and warmest period, earthworm population was reduced (see Fig. 2). Crumsey et al. (2013) also showed that soil water content had significant effects on the relative abundance of earthworm species and was a stronger driver of earthworm species richness than pH or soil organic carbon. Overall, earthworm population density and biomass values are similar to those reported by Ewing et al. (2015). Furthermore, we also observed differences in earthworm diversity with more abundance of epigeic compared to anecic and endogeic earthworms. This may be related to the deep burrowing ability of anecic and endogeic earthworms, a hypothesis supported by previous studies (Jégou et al. 2001; Uvarov 2009). This could also partly explain the low dispersal rates observed of these anecic and endogeic earthworms in the topsoil (0–20 cm) under different tree species and seasons, because of their activity in the lower layers of soil.

Similar to our findings, Suthar (2012) showed that maximum earthworm abundance occurs in autumn and declines sharply in winter. Low physiological activity and winter temperatures are likely to limit earthworm activity. In subsequent spring, earthworms reappeared and showed a moderate population density, which again declined in early summer and virtually disappeared in winter. In the rainy season, earthworms reappeared in topsoil layers and newly hatched subadults were also recorded during the period. The population abundances observed during rainy season showed continuous increase up to autumn months (Suthar 2012). Among the factors responsible for earthworm distribution in this study, soil moisture content seems to be of major importance as it has been observed in other studies (Edwards and Bohlen 1996; Crumsey et al. 2014). Kaushal

Table 2 Mean (±standard error) soil water content and chemical features under different tree species [*C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC)] and months in the topsoil (0–20 cm depth)

Months	Tree species	Water content (%)	pH	Organic C (%)	Total N (%)	C/N ratio
April	CB	34.2 ± 1.920b	6.29 ± 0.018ab	1.11 ± 0.257b	0.17 ± 0.021bc	21.60 ± 1.427a
	UM	36.2 ± 1.911b	6.11 ± 0.100bc	1.20 ± 0.235b	0.16 ± 0.030bc	8.29 ± 1.866b
	PF	41.5 ± 1.603b	6.08 ± 0.077bc	1.48 ± 0.204b	0.15 ± 0.024bc	12.87 ± 5.401b
	AG	39.6 ± 2.010ab	6.23 ± 0.028abc	1.24 ± 0.092b	0.25 ± 0.025a	5.02 ± 0.398b
	PC	35.5 ± 3.817b	5.99 ± 0.138c	1.18 ± 0.048b	0.22 ± 0.020ab	5.77 ± 0.800b
	QC	44.1 ± 3.623a	6.36 ± 0.015a	2.88 ± 0.285a	0.12 ± 0.011c	25.12 ± 2.7106a
	Mean	38.5 ± 1.177A	6.180 ± 0.037A	1.51 ± 0.136B	0.17 ± 0.012C	13.1 ± 1.746
June	CB	27.4 ± 0.676	5.97 ± 0.053ab	1.34 ± 0.219b	0.31 ± 0.042ab	3.56 ± 0.522b
	UM	27.4 ± 1.523	6.09 ± 0.019a	1.79 ± 0.365b	0.22 ± 0.022bcd	8.21 ± 1.675b
	PF	31.7 ± 0.609	5.91 ± 0.005b	2.01 ± 0.182b	0.18 ± 0.029cd	12.51 ± 2.204b
	AG	28.7 ± 1.425	6.09 ± 0.054a	1.44 ± 0.042b	0.39 ± 0.074a	4.27 ± 0.758b
	PC	29.1 ± 1.078	5.89 ± 0.037b	1.46 ± 0.069b	0.30 ± 0.008abc	4.88 ± 0.193b
	QC	29.1 ± 0.799	6.09 ± 0.035a	3.12 ± 0.537a	0.14 ± 0.030d	32.11 ± 1.890a
	Mean	28.9 ± 0.482C	6.01 ± 0.021C	1.86 ± 0.156AB	0.25 ± 0.021AB	10.9 ± 2.950
August	CB	22.0 ± 1.344c	6.18 ± 0.009a	1.65 ± 0.282b	0.34 ± 0.061ab	5.17 ± 0.781b
	UM	22.0 ± 0.260c	6.11 ± 0.032ab	1.82 ± 0.328b	0.26 ± 0.022bcd	7.12 ± 1.086b
	PF	26.4 ± 418ab	5.99 ± 0.020d	2.37 ± 0.346b	0.22 ± 0.033cd	11.98 ± 3.110b
	AG	27.5 ± 1.575a	6.12 ± 0.015abc	1.80 ± 0.226b	0.44 ± 0.039a	4.15 ± 0.513b
	PC	23.7 ± 1.432bc	6.09 ± 0.021c	1.57 ± 0.221b	0.32 ± 0.032bc	5.28 ± 1.369b
	QC	25.5 ± 0.928ab	6.16 ± 0.014ab	3.17 ± 0.047a	0.15 ± 0.172d	33.3 ± 6.636a
	Mean	24.54 ± 0.566D	6.11 ± 0.01B	2.06 ± 0.141A	0.29 ± 0.021A	11.2 ± 2.220
October	CB	33.8 ± 2.587	5.96 ± 0.041	1.51 ± 0.207b	0.25 ± 0.036ab	18.9 ± 2.437b
	UM	36.7 ± 2.825	5.99 ± 0.008	1.57 ± 0.221b	0.19 ± 0.009bc	8.31 ± 1.553c
	PF	34.4 ± 1.010	5.93 ± 0.017	1.68 ± 0.246b	0.21 ± 0.027abc	8.31 ± 0.684c
	AG	39.1 ± 1.099	5.91 ± 0.024	1.55 ± 0.161b	0.32 ± 0.062a	5.51 ± 0.938c
	PC	34.8 ± 1.529	5.92 ± 0.012	1.53 ± 0.147b	0.28 ± 0.043ab	6.00 ± 1.092c
	QC	37.6 ± 1.785	5.94 ± 0.035	2.96 ± 0.249a	0.12 ± 0.025c	27.71 ± 3.770a
	Mean	36.07 ± 0.797B	5.94 ± 0.010D	1.80 ± 0.124AB	0.23 ± 0.018BC	12.5 ± 1.694

Different letters indicate a significant difference under the six tree species (lowercase letters) and month (capital letters) (Duncan's test; $P < 0.05$). Values are the mean ($n = 5 \pm 1$ SE for each tree species, N total = 30; $n = 30 \pm 1$ SE for each month, N total = 120)

and Bisht (1994) found that in northern part of India, the population density of earthworm was much higher during the wet period of the year than dry months. In Indian subtropical climate in plains, the abundance of earthworm population has been recorded during the rainy season leading to maximum densities in September–October and least during the hot summer (May–June) (Suthar 2012). In agreement with our results, Suthar (2012) indicated that earthworm population increased during spring and autumn but decreased in summer and winter. Besides moisture content, some other soil properties affected earthworm population at our study site. The differences in earthworm density/biomass in the present study can be also related to differences in soil fertility (Emmerling and Strunk 2012; Sigurdsson and Gudleifsson 2013; Sackett et al. 2013). In addition, in April, higher soil pH may have favored earthworm population according to previous studies (Whalen and Costa 2003; Chaudhuri and Paliwal 2008; Valckx et al. 2009). As suggested by Nurhidayati et al. (2012), earthworm abundance in the soil in spring is greatly related to food availability and soil physicochemical properties such as soil moisture, soil temperature and soil pH.

Soil microbial respiration

Soil respiration is a good indicator of total belowground allocation of carbon and of ecosystem productivity (Davidson et al. 2000). Similar to earthworm density/biomass, soil microbial respiration was not affected by tree species and showed similar temporal trends in all soils under different tree species with maximum activity in

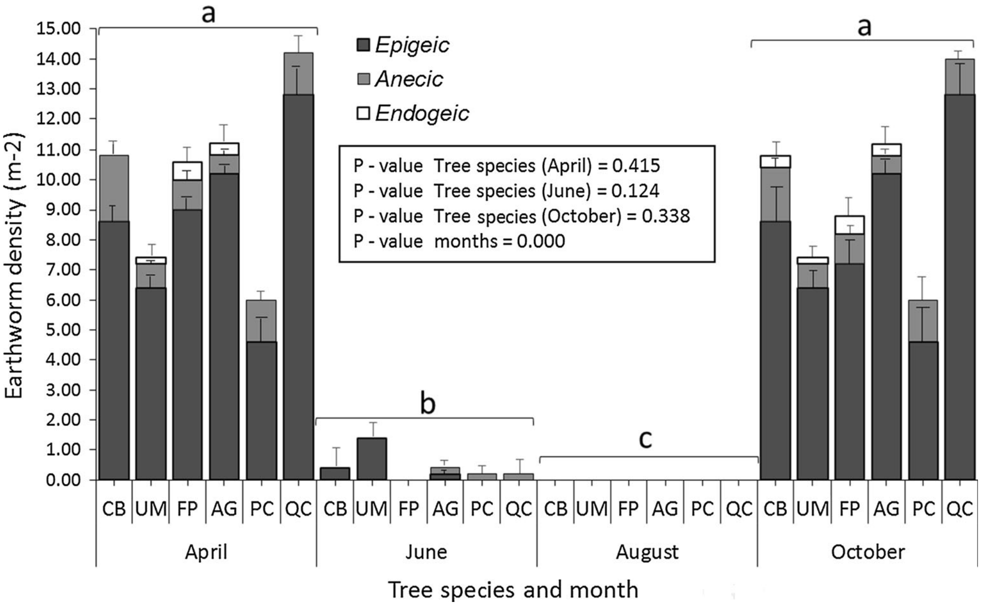


Fig. 3 Temporal variability in earthworm population density (epigeic, anecic and endogeic) (m^{-2}) under the six tree species studied [*C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC)] in the 4 months. *Different letters* (*a*–*c*) indicate a significant difference under the six tree species and month (analysis of variance (ANOVA) and Duncan's test; $P < 0.05$). Values are the mean ($n = 5 \pm 1$ SE for each tree species, N total = 30; $n = 30 \pm 1$ SE for each month, N total = 120)

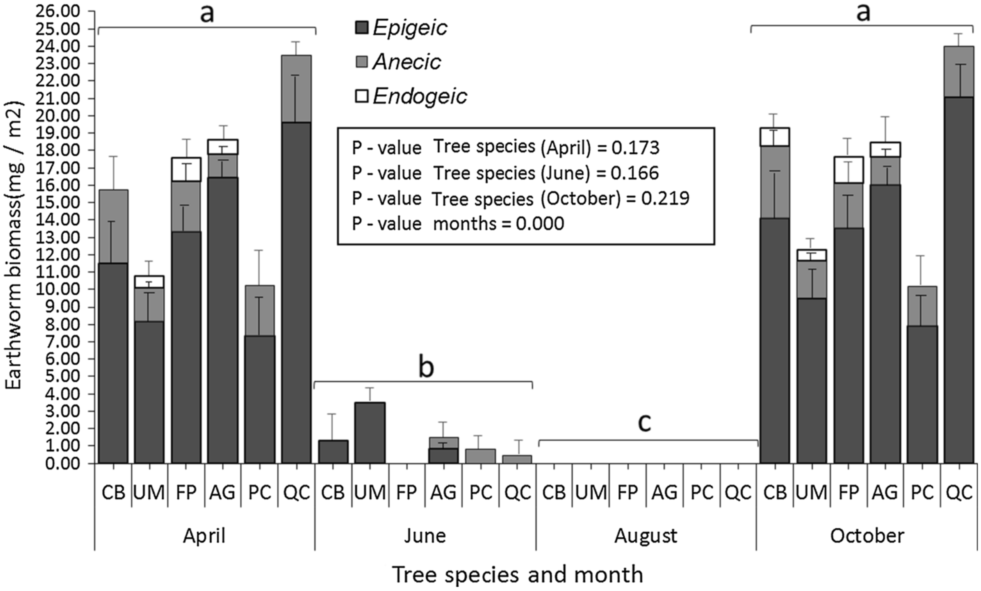


Fig. 4 Temporal variability in earthworm population biomass (epigeic, anecic and endogeic) (mg m^{-2}) under the six tree species studied [*C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC)] in the 4 months. Different letters (a, b and c) indicate a significant difference under the six tree species and month (analysis of variance (ANOVA) and Duncan's test; $P < 0.05$). Values are the mean ($n = 5 \pm 1$ SE for each tree species, N total = 30; $n = 30 \pm 1$ SE for each month, N total = 120)

spring and summer, particularly under AG tree species. According to the findings of Mo et al. (2004) and Kooch et al. (2016), soil total N content is the main factor affecting decomposition of plant litter, and litter decomposition rate is a major source of soil CO_2 emission (Rey et al. 2002) in the summer with high temperatures. Furthermore, the activities of these microorganisms can continue for a short time in the casts because of the suitable amount of soluble carbon and nutrient resources (Zirbes et al. 2012). Greatest values of total N and microbial respiration were found under AG species compared to other tree species. Parallel to our study, Cao et al. (2011) reported that the soil N content promoted the release of CO_2 from soils since better litter quality promotes microbial litter decomposition. Indeed, (in spring season with suitable soil moisture and temperature), the survival of microorganisms in the earthworm gut depends on their capacity to resist digestive enzymes of microbial or earthworm origins, or to bacteriostatic and microbial substances and transit time. For example, epigeic species, which feed on rich substrates, need a complex enzymatic system (Zirbes et al. 2012). Zirbes et al. (2012) have shown that some microorganisms of soil increase in abundance through the gut tract of earthworms.

In our study, environmental variables controlled soil microbial respiration activity with no differences between tree species. Similar to our findings, previous studies (Schlesinger and Andrews 2000; Liu et al. 2012; Luo et al.

Fig. 5 Temporal variability in soil microbial respiration (mg CO_2–C g dry soil^{-1} day^{-1}) under the six tree species studied [*C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC)] in the 4 months. *Different letters* (a, ab, b, abc, bc and c) indicate a significant difference under the six tree species and month (analysis of variance (ANOVA) and Duncan's test; $P < 0.05$). Values are the mean ($n = 5 \pm 1$ SE for each tree species, N total = 30 for all trees; $n = 30 \pm 1$ SE for each month, N total = 120 for all month)

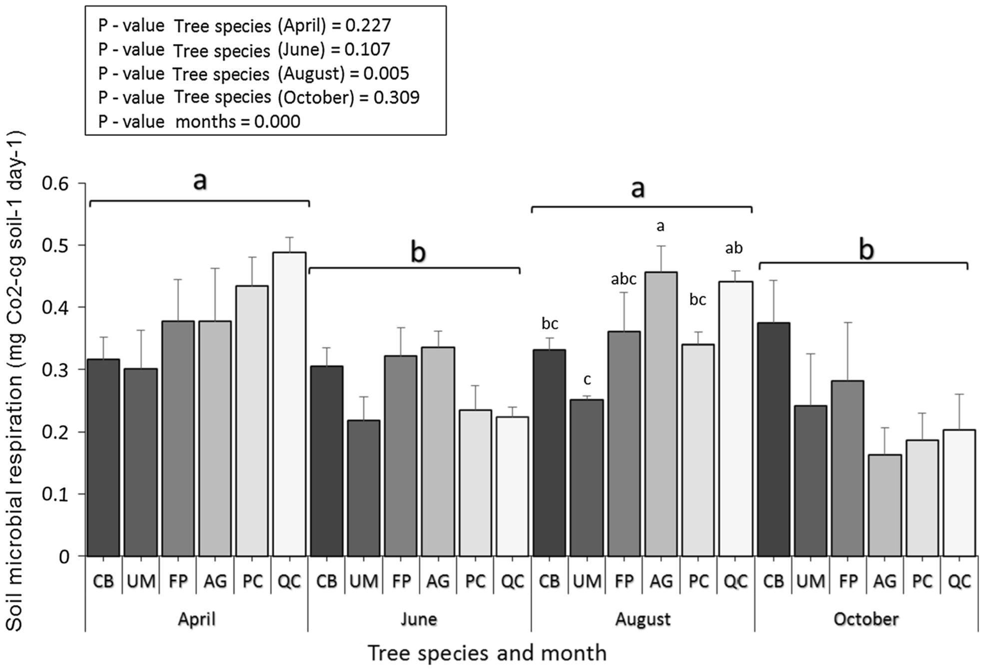
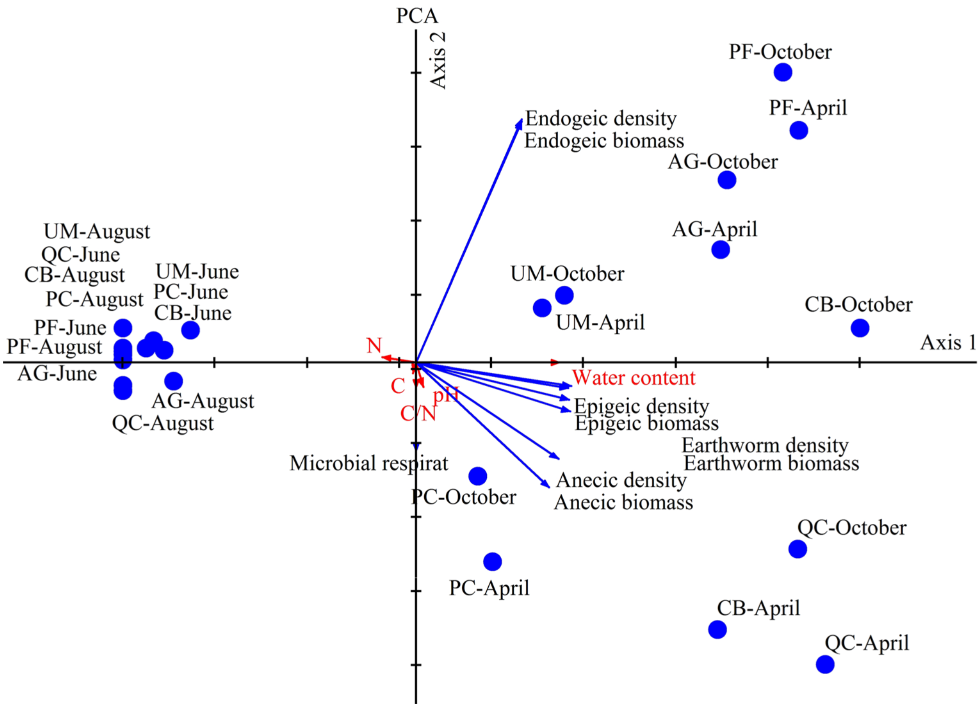


Fig. 6 PCA based on the correlation matrix of the tree species, soil water content, chemical and biological properties in different months. The studied tree species were the *C. betulus* (CB), *U. minor* (UM), *P. fraxinifolia* (PF), *A. glutinosa* (AG), *P. caspica* (PC) and *Q. castaneifolia* (QC). PC1: Eigen value = 6.20, percent of variance = 68.94, cumulative percent of variance = 68.94; PC2: Eigen value = 1.36, percent of variance = 15.12, cumulative percent of variance = 84.06



2014) have observed higher values of soil microbial respiration in the summer than autumn season in temperate regions where most favorable environmental conditions occur. In our study area, temperature increases during summer season (see Fig. 2) favoring microbially mediated processes in soils (Kooch et al. 2015). Accelerated turnover of soil organic compounds in summer is supported by increased organic C and total N, as well as by microbial respiration. Particularly, strong increases were observed for soil microbial respiration with increasing pH (in April), organic C and total N (in August). Variability in soil chemical properties over the season as well as changes in soil moisture can be responsible for temporal changes in soil microbial respiration. In August and April months, increased pH (Cookson et al. 2006), higher C (Kuzyakov et al. 2000; Huang et al. 2014; Makita and Fujii 2015) and

N (Mo et al. 2004; Werner et al. 2007; Wang et al. 2013) and more available nutrients due to greater earthworms mass (Ponge 2003; Zirbes et al. 2012) can contribute to microbial respiration.

Conclusion

This study shows that tree species in the mixed forest present the same earthworm populations (i.e., earthworm density/biomass) and microbial respiration despite affecting soil chemical properties. In this temperate mixed forest, seasonal environmental changes drive soil biological activity being highest in spring and summer when optimum environmental conditions occur in this region. The main soil properties affecting soil biological activity were soil water content, pH, total organic C and total N. Our study shows that deciduous tree species in the mixed forest affect soil chemical properties and contribute to spatial heterogeneity, but biological activity is driven by climate. Therefore, expected changes in climate will potentially have a stronger impact on soil biological activity than changes in forest diversity.

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