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Earthworms – good indicators for forest disturbance

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ABSTRACT
In temperate forests, formation of canopy gaps by windthrow is a characteristic natural disturbance event. Little work has been done on the effects of canopy gaps on soil properties and fauna, especially earthworms as ecosystem engineers. We conducted a study to examine the reaction of earthworms (density/biomass) and different soil properties (*i.e.*, soil moisture, pH, organic matter, total N, and available Ca) to different canopy gap areas in 25-ha areas of Liresar district beech forest located in a temperate forest of Mazandaran province in the north of Iran. Soil samples were taken at 0-15, 15-30 and 30-45cm depths from gap center, gap edge and closed canopy using core soil sampler with 81cm^2 cross section. The earthworms were collected simultaneously with the soil sampling by hand sorting method. Our study supports that the canopy gap will create a mosaic of environmental conditions. Earthworm's density and biomass tended to be higher in small canopy gaps compared with the other canopy gap areas. Earthworm's population showed decreasing trend from closed canopy to disturbed sites (gap edge and gap center). The top soil was more appropriate to presence of earthworms although ecological groups have occupied different soil layers. As a conclusion, earthworms can be introduced as good bio-indicator of environmental changes that occur by disturbance.

Key words: Beech forest, canopy gap, soil properties, earthworm density and biomass

Introduction

Soil fauna, especially earthworms, are considered to be important components of the soil ecosystem for maintaining nutrient cycling and biological soil fertility (Osler & Sommerkorn, 2007). Soil fauna are thought to be useful indicators of soil quality because they are sensitive to changes in land management and are involved in many soil functions (Yeates, 2003). As indicators of soil quality, the abundance and diversity of soil fauna integrate physical, chemical and microbiological properties of soil, and reflect general ecological change (Menta et al., 2008; Paolo et al., 2010). It remains unclear; however, which soil fauna are the best indicators of soil quality, and what soil fauna data should be used to assess soil quality. Earthworms have been viewed as ecological engineers (Cole et al., 2006; Bartlett et al., 2009) and have many impacts on soil properties (Edwards, 2004; Groffman et al., 2004). Earthworms have been shown to affect soil physical properties, such as density, structure, aeration, and moisture (Ponder et al., 2000). Chemical properties, such as pH, nutrient availability, and heavy metals are affected by earthworms (Wen et al., 2006). Earthworms influence microbial composition, biomass, and activity and thus affect the rates and patterns of mineralization and immobilization in soils (Binet et al., 1998). Earthworm activity is influenced by many factors, such as food quality and quantity (Curry, 2004), temperature and moisture (Berry & Jordan, 2001), soil properties (*e.g.*, pH, texture and structure) (Baker & Whitby, 2003), and multitude of biotic interactions (*e.g.*, competition, predation, parasitism, disease, etc.) (Edwards, 2004).

Soil processes are controlled by a set of relatively independent state factors including climate, organisms, relief, parent material and time, and by a group of interactive controls such as disturbance regime and human activities (Scharenbroch & Bockheim, 2007a, 2007b). Disturbance is ubiquitous in forest ecosystems. Defined as "any relatively discrete event in time that disrupts ecosystems, community or population structure and changes resources, substrate availability, or the physical environment", disturbance determines forest species composition, structure, and process. Furthermore, disturbances exert their influence over a wide range of temporal and spatial scales. This disturbance, in turn, promotes changes in resource fluxes, changes that lead to some form of reorganization of the disturbed patch or gap at structural and functional levels that may be similar or dissimilar to pre-disturbance levels. Resource levels and inputs are changed and species respond accordingly (Jonasova et al., 2010).

In Iran beech forests, formation of gaps by windthrow is a characteristic natural disturbance event. Gap size varies greatly from the size of only a single crown to vast open fields with diameters of many tree lengths. However, changes in abiotic and biotic conditions depend both on gap size and within-gap position (Kwit & Platt, 2003). Consequently, it is not easy to predict how soil properties react to gap formation. Disturbances caused by canopy gaps received much attention in the last decades and they are regarded as important factors in forest dynamics. Canopy openings as a result of tree falls create an environment different from the adjacent forest, which influences plant regeneration. In addition, gap processes partly determine forest structure and play an important role to maintain plant species richness. Thus, the creation of gaps in forests is an opportunity for the system to change in both species dynamics and ecological processes (Muscolo et al., 2007a, 2007b).

It is now well-known that forest disturbances generally result in significant variability of earthworm populations in temperate landscapes (Kooch & Hosseini, 2010) with significant ecological implications for ecosystem functioning (Gonzalez et al., 2003). Consequently, earthworms should serve as good indicators of environmental changes that occur by disturbance. Relationships of earthworms and soil properties have been well-documented for forest ecosystems (Whalen, 2004; Marhan & Scheu, 2005; Heneghan et al., 2007). Up to now, however, only few studies (Haynes et al., 2003; Dlamini & Haynes, 2004) have simultaneously studied changes in earthworm communities and soil parameters, in response to forest disturbance. It is not clear whether earthworm populations are mainly controlled by the amount of food, its quality, or the chemical properties of their environment (Aubert et al., 2003; Scheu et al., 2003; Gonzalez et al., 2003). Therefore, determining the relation among earthworm's density and biomass with different areas of canopy gaps and edaphic conditions are essential for management of forest ecosystems. The present study is intended to assessing of canopy gap areas on soil physico-chemical properties, earthworm density and biomass in hyrcanian forests of Iran that is the first survey in these forests.

Materials and Methods

Study area

This research was performed in the Liresar Watershed Forest Management Plan, which is one of the oldest forest management plans of Hyrcanian forest of Iran, which is active during recent 50 years (Avatefi Hemmat et al., 2012). These forests located in the Mazandaran province in north of Iran (Figure 1) with the area of 6500 ha, 39˚ 36′ N, and 50˚ 51′ E. The maximum elevation is 3433 m and the minimum is 820 m. Rainfall distribution is related to the seasons and based on the information from long term data, about 35-45% of the rainfall will occurred in autumn season (from September to November), 18-35% in winter season (December to February), and having the rest of the rainfall (10-20%) in summer season (June to August). The region has relatively mild temperature and the difference between maximum and minimum temperature during a year is about 30°C. The average of minimum temperature from December to March is below zero. The parent materials are alluvial sediments remaining from 4th period of Geochronology, which have a low slope degree without any gravel; Bedrock is limeston-dolomite with silty-clay-loam soil texture (Mousavi et al., 2012). These forests are covered mostly with *Fagus orientalis* Lipsky (Oriental Beech) mixed with *Carpinus betulus* L. (Common Hornbeam).

Soil sampling and analysis

In the present study, 25-ha area of Liresar district beech forest were considered. Geographical position and all of canopy gaps were recorded by Geographical Position System (GPS). Gaps required a minimum canopy opening of 30 m^2 and trees growing in the gap to be less than two thirds the height of the closed adjacent forest (Runkle, 1992). Canopy gaps areas were measured in the field according to Runkle (1992). Sampling protocol was built up by locating and measuring two perpendicular lines in each gap: one along the

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longest line visible and one perpendicular to it at the widest section of the gap. In this research, 20 canopy gaps (among 4 area classes with 5 replications for each) were selected to study. The canopy gaps were categorized into four classes which are as follows: small gaps with mean area = 100.23 m^2; medium gaps with mean area = 310.31 m^2; large gaps with mean area = 518.36 m^2 and very large gaps with mean area = 711.73 m^2.

For this purpose, three positions were distinguished including gap center, gap edge and closed canopy. Soil samples were taken at 0-15, 15-30 and 30-45cm depths from all positions using core soil sampler with 81cm^2 cross section. Roots, shoots and pebbles in each sample were separated by hand and discarded. The air-dried soil samples were sieved (aggregates were crushed to pass through a 2 mm sieve) to remove roots prior to chemical analysis. The soil moisture measured by drying the soil samples at 105°C for 24 hours. The pH was determined by an Orion Ionalyzer (Model 901) pH meter in a soil: water solution of 1:2.5 ratios and the organic C using the Walkey-Black technique (Allison, 1975). The total N was measured using a semi Micro-Kjeldahl technique (Bremner & Mulvaney, 1982). The amounts available of Ca (by ammonium acetate extraction at pH 9) were determined with an atomic absorption spectrophotometer (Bower et al., 1952). The earthworms were collected during the soil sampling by hand-sorting and biomass was measured at the laboratory (Edwards & Bohlen, 1996). Ecological groups of earthworms (epigeic, anecic and endogeic) were identified by external characteristics using the key of BOUCH (Kooch & Hosseini, 2010).

Statistical analysis

Normality of the variables was checked by Kolmogrov-Smirnov test and Levene test was used to examine the equality of the variances. Differences between canopy gaps areas, positions and depths in soil properties were tested with three-way analysis (ANOVA) using the General Linear Model (GLM) procedure. Interactions between independent factors were tested also. Duncan test was used to separate the averages of the dependent variables which were significantly affected by treatment. Significant differences among treatment averages for different parameters were tested at $P \leq 0.05$. Nonparametric Kruskal-Wallis analysis of variance was used to find differences in earthworm's density and biomass of treatments, because in some cases there was no homogeneity of variance. Analyses of whole data were done in SPSS Ver. 11.5 of statistical program.

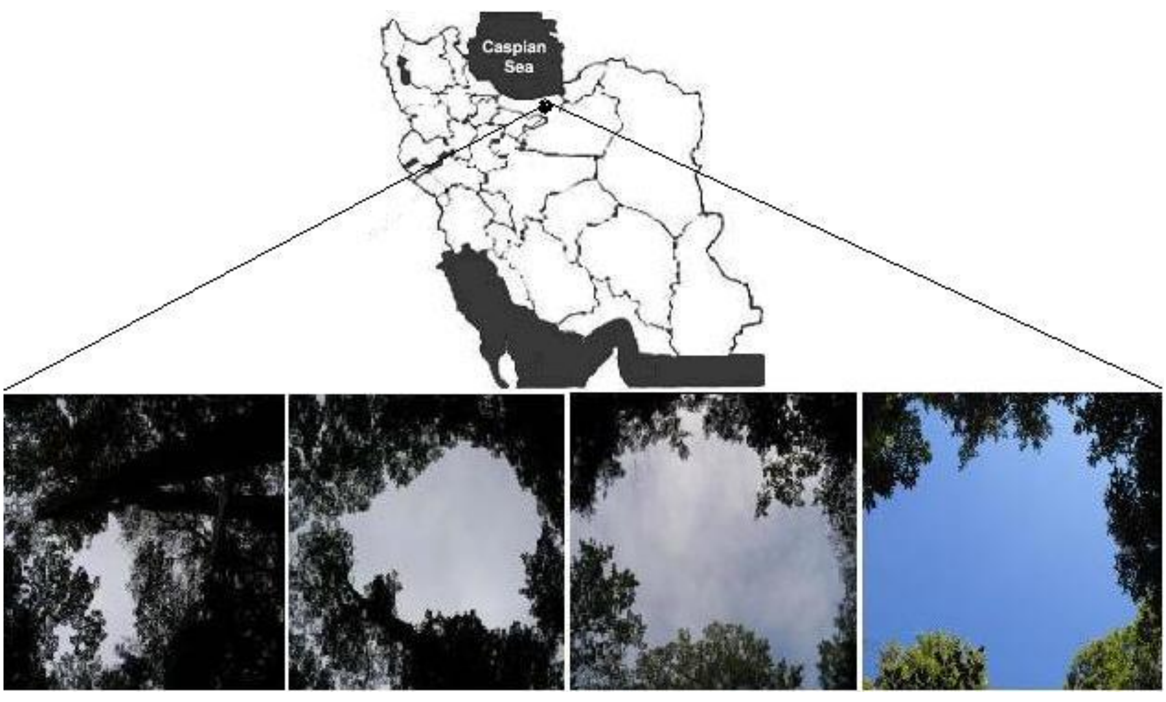

Figure 1. *Location of the study area in the Liresar region, with photo of canopy gaps (figure not to scale)*

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Results

Soil moisture was significantly greater in small gap (38.50%), closed canopy (41.96%) position and top soil (32.35%) than in the other treatments (Table 1). Soil pH was significantly higher in very large gaps (6.82) in comparison to small, medium and large gaps (Table 1). The highest value of this character was detected in gap center (6.53) position and 30-45 cm (6.82) soil depth (Table 1). Organic matter increased with increasing size of the gaps (4.31% - 4.60%), decreased from gap center to closed canopy (4.32% - 4.08%) and soil depth (4.39% to 4.09%) (Table 1). Compare mean of total N indicated that large gaps (0.19%) had the higher amounts than in the other gap size. Gap center (0.18%) position and 0-15cm (0.18%) soil depth had the greatest value of this character in comparison to the other positions and depths (Table 1). Greater amounts of carbon to nitrogen ratio were found in small gap (17.17), closed canopy (18.52) position and 30-45 cm (18.28) soil depth, significantly (Table 1). Greater amounts of available Ca were detected in medium (12.37 mg/kg) and large (13.29 mg/kg) gaps, gap center (12.64 mg/kg) position and 0-15cm (12.41 mg/kg) soil depth (Table 1).

Analysis of data showed that density and biomass of earthworm's ecological groups had significant differences among gap areas, positions and soil depths (Table 2). Earthworm’s (epigeic, anecic and endogeic) density and biomass tended to be higher in small gaps compared with the other gap size (Table 2). The population of earthworms showed decreasing trend from closed canopy to disturbed sites, *i.e.* gap edge and gap center (Table 2). The top soil was more appropriate to presence of earthworms although ecological groups have occupied different soil layers (Table 2).

Discussion

Disturbances caused by canopy gaps received much attention in the last decades and they are regarded as important factors in forest dynamics. Following canopy opening, it is assumed that the increased levels of light and nutrients (Gagnon et al., 2003) in gaps act as important factors controlling natural regeneration, which depends upon many other variables, including biological, chemical and physical properties of the seedbed (Muscolo et al., 2007a).

Table 1. *Mean of soil physico-chemical properties in gap areas, positions and soil depths*

Variable /	soil character	Moisture (%)	pH	Organic matter (%)	Total N (%)	Carbon to nitrogen ratio	Available Ca (mg/kg)
Gap area	Small	38.50±1.22a	6.73±0.08d	4.31±0.04d	0.12±0.01d	17.17±0.08a	12.09±0.07c
	Medium	33.83±1.08b	6.76±0.09c	4.36±0.02c	0.13±0.02c	16.54±0.05b	12.37±0.02b
	Large	28.20±1.09c	6.80±0.07b	4.56±0.02b	0.19±0.00a	15.83±0.05c	13.29±0.00a
	Very large	26.03±2.06d	6.82±0.07a	4.60±0.01a	0.17±0.00b	16.23±0.06c	11.83±0.02c
	F - value	205.80**	171.96**	164.32**	58.53**	7.53**	70.92**
Gap position	Center	25.03±1.23c	6.53±0.06a	4.32±0.02a	0.18±0.00a	14.47±0.07c	12.64±0.06a
	Edge	27.93±1.67b	6.41±0.09b	4.12±0.01b	0.15±0.01b	16.32±0.02b	11.82±0.04b
	Closed canopy	41.96±2.12a	6.30±0.04c	4.08±0.00c	0.10±0.00c	18.52±0.04a	10.53±0.00c
	F - Value	708.69**	894.80**	769.74**	455.45**	126.93**	167.57**
Soil depth (cm)	0 - 15	32.35±1.28a	6.78±0.09b	4.39±0.00a	0.18±0.00a	14.72±0.05c	12.41±0.01a
	15 - 30	32.15±2.09a	6.79±0.05b	4.27±0.07b	0.15±0.00b	16.31±0.03b	11.73±0.00b
	30 - 45	30.42±1.78b	6.82±0.07a	4.09±0.01c	0.14±0.01c	18.28±0.03a	11.14±0.00c
	F - value	9.77**	8.48**	69.47**	199.14**	98.20**	81.90**
Interactions F-value	Area × position	27.34**	60.03**	41.33**	26.09**	3.63**	46.95**
	Area × soil depth	8.19**	3.13**	1.00 ns	1.54 ns	0.59 ns	1.66 ns
	Position × soil depth	18.66**	2.03ns	0.15 ns	6.01**	2.42*	0.73 ns
	Area × position×soil depth	4.78**	2.50**	0.32 ns	2.30*	0.17 ns	1.29 ns

** Difference is significant at the 0.01 level. *Difference is significant at the 0.05 level. (ns): Non significant differences ($P > 0.05$).
Values are the means ±SE of the mean. Within the same column the means followed by different letters are statistically different ($P < 0.05$).

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Table 2. *Kruskal-Wallis analysis for earthworm's density and biomass*

Variables / earthworm groups		Epigeic Density (n/m^2)	Epigeic Biomass (mg/m^2)	Anecic Density (n/m^2)	Anecic Biomass (mg/m^2)	Endogeic Density (n/m^2)	Endogeic Biomass (mg/m^2)
Gap area	Small	2.73	0.28	5.12	0.52	6.32	0.62
	Medium	1.98	0.10	4.38	0.35	5.12	0.49
	Large	0.62	0.06	2.15	0.20	3.13	0.38
	Very large	0.19	0.01	1.25	0.11	1.56	0.14
Statistical characters	Chi square	9.52	9.70	6.82	6.97	12.95	12.32
	DF	3	3	3	3	3	3
	Sig.	0.02*	0.02*	0.07 ^{ns}	0.07 ^{ns}	0.00**	0.00**
Gap position	Center	0.02	0.14	0.06	0.58	0.09	0.85
	Edge	0.17	1.28	0.29	2.39	0.42	5.12
	Closed canopy	0.26	2.32	0.63	5.22	0.73	7.29
Statistical characters	Chi square	7.74	7.98	26.63	26.80	25.22	25.19
	DF	2	2	2	2	2	2
	Sig.	0.02*	0.01*	0.00**	0.00**	0.00**	0.00**
Soil depth (cm)	0 - 15	0.39	3.15	0.13	1.12	0.05	0.52
	15 - 30	0.00	0.00	0.58	5.98	0.08	0.88
	30 - 45	0.00	0.00	0.00	0.00	0.86	9.12
Statistical characters	Chi square	30.73	30.69	40.27	38.49	51.68	51.34
	DF	2	2	2	2	2	2
	Sig.	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**

** Difference is significant at the 0.01 level. *Difference is significant at the 0.05 level. (ns): Non significant differences ($P > 0.05$).

Many gap studies focus on vegetation dynamics and effects on microclimatic, but few studies have addressed below-ground effects of gaps (Muscolo et al., 2007a, 2007b). Regarding to the variability of soil properties following creation of canopy gap (Kooch et al., 2012) we are suspected to introduce the earthworm as good indicator of forest disturbance. According to previous studies (Kooch et al., 2014a, 2014b) the earthworm's population are imposed by canopy opening directly or by changed soil properties, indirectly. As dominant species in the study area, beech twigs and leaves are need to longer times for decomposition. This character with old age of forest stands are due to increasing of litters volume in understory (Huttl et al., 2000). A thick humus layer creates appropriate positions for moisture preservation in forest lands. Sponge character under closed canopy will increase moisture preservation capacities in comparison with the canopy opening (Gray et al., 2002; Ritter & Vesterdal, 2006). With considering to superficial rooting system of beech, thus we are suspect to use of beech living roots from gap within moisture. Similar finding was also reported by Muth & Bazzaz (2002) in a study of mixed hardwood forest. Galhidy et al. (2006) claimed that even centers of small gaps are beyond the reach of the root system of surrounding trees. Thus, less amounts of soil moisture were considered in within gap position similar to our findings in this research.

Soil acidification often occurs with nitrate leaching and nitrification; thus, it is unlikely gap disturbance have a role in acidification of these forest soils through nitrate leaching or increased nitrification (Scharenbroch & Bockheim, 2007a). Small gaps tended to have lower amounts of soil pH that can be related to presence complexes of sustain organic acids as in gaps with more openings these complexes are leaching from soil upper layers. Thus, gap larger areas tended to have higher pH. Similar status can be considered in different positions of gaps as in gap center leaching of acid complexes more occurred and soil pH is increased. Scharenbroch & Bockheim (2007a) detected no significant differences in soil pH character for gap different areas. The higher amounts of pH in soil deeper layers can be related to lower values of organic matter in soil beneath depths as inversely relation found between these characters (Kooch et al., 2012; Kooch et al., 2014a, 2014b). Density and particle size separations isolate district soil organic matter pools for relating stabilization and turnover of carbon in soil (Six et al., 2002). Soil organic matter associated with silt and clay particles are considerably more recalcitrant, with turnover times ranging from 400 to 1000 years (Buyanovsky et al., 1994). Soil upper layers had more organic matter contents regarding near to litter sources and plant residuals. However, gap dynamics may be important in explaining discrepancies in theories suggesting old-growth forests are inconsequential carbon sinks or are actively accumulating carbon (Zhou et al., 2006). According to Scharenbroch & Bockheim (2007a) findings the canopy gaps effects on soil organic matter character was non-significant.

Scharenbroch & Bockheim (2008) claimed that canopy gaps are susceptible to N leaching less, thus the amounts of this character is less in opening areas soil than in closed canopy. Changes in N uptake, microclimate (*e.g.*, increased radiation, temperature and moisture), and substrate associated with gaps effect significant influence on forest N dynamics. In general, increased organic matter decomposition and N mineralization and reduced root nitrogen uptake tends to favor leaching of inorganic N in gaps relative to the undisturbed closed forest (Denslow et al., 1998). Increased N concentration in the gaps may partly be attributed to a lack of N uptake by regeneration or ground vegetation in the early years after gap formation, as also found by Knight et al. (1991). Ritter & Vesterdal (2006) also pointed in the advanced regeneration in the seventh year after gap formation was still not enough to reduce N concentrations significantly. We suspect that a long-time is need to reduction of N concentrations in within gaps, significantly. In general, solar radiation will increased with increasing of canopy opening areas that is due to accelerating decomposition of litters. But if the opening be very large, decrease in base cations, like Ca, in gaps is likely a result of leaching losses. Scharenbroch & Bockheim (2007a) reported that the leaching is the most important reason for decrease of base cations in within gaps. Their results suggest an increased nutrient leaching potential as a result of relatively large (300-2000 m^2) gaps in old growth northern hardwood-hemlock forests. The results of our research is indicating that base cations leaching potential increased with expanding of canopy opening areas from medium to large; thus soil is poor of nutrient elements in large canopy gaps. This important should be considered in forest management and trees marking for utilization to prevent of gaps formation with large opening areas.

Regarding to variability of different soil properties following the canopy opening the earthworm populations were changed in different conditions. It is well-known that earthworm distribution and biomass are affected by changes of soil properties (*e.g.*, moisture, pH, nutrient availability, etc.) (Kooch et al., 2012). Earthworm density and biomass were significantly greater in small gaps, closed canopy position and soil upper layers. Different soil properties could be effective on earthworm populations. The most important effective factors can be soil higher moisture and lower temperature in small gaps (Kooch, 2012). Earthworms tended to be less within gaps, likely due to decreased soil moisture and increased temperature in gaps. Earthworm activity is quite sensitive and tends to decrease with low moisture contents and high temperatures (Nachtergale et al., 2002). Thus, earthworm density and biomass is decreased with increasing of canopy cover opening areas by reason of soil moisture reduction and increase of temperature. As similar, closed canopy position has greater soil moisture and less temperature in compare to the other positions. Therefore, this position created more appropriate condition for gathering of earthworms. Gap edge has medium condition for assemblage of earthworms than to gap center and closed canopy. Earthworms (especially endogeic) are able to migration more beneath layers and avoid of soil drought, especially in summer season (Hale & Host, 2005).

Conclusion

In temperate forests, formation of gaps by windthrow is a characteristic natural disturbance event. Our study supports that the canopy gap will create a mosaic of environmental conditions. It is now well-known that forest disturbances generally result in significant variability of earthworm populations in temperate landscapes with significant ecological implications for ecosystem functioning. Consequently, earthworms can be introduced as good bioindicator of environmental changes that occur by disturbance.

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