

**Using Soil Stable Isotopes,  $\delta^{13}C$  and  $\delta^{15}N$ , Properties for Interpreting Effects of Forest Understory Vegetation Removal on Nutrient Cycling** **$\delta^{13}C$  ve  $\delta^{15}N$  Durağan İzotoplar Aracılığıyla Diri Örtü Kontrolünün Besin Döngüsüne Etkisinin Belirlenmesi****Okta YILDIZ<sup>1</sup>****Abstract**

Forest harvesting and understory vegetation management may disturb the ecological integrity of forest ecosystems. Abrupt change in plant composition in the stand also modifies the nutritional status of the site. The aim of this study is to analyze the soil stable isotopes,  $\delta^{13}C$  and  $\delta^{15}N$  properties for interpreting effects of clearing understory vegetation on nutrient cycling. The study utilized a previous project in which the understory vegetation was variably cleared in Douglas-fir plantations situated in the Pacific Coast of Oregon, USA. Treatments included removing brush and herbaceous vegetation control at varying ratios. Also, a control plot was included in the experiment where no vegetation removal (DFC) was employed. On one of the plots, shrubs were completely cleared, leaving only herbs and Douglas-fir (DFH). Another plot received complete removal of shrubs and herbs, leaving only Douglas-fir (DFO). Soil samples were collected on each plot at 5<sup>th</sup> and 15<sup>th</sup> year of the stand establishment. Soil was sub-sampled to distinguish light (LF)- and heavy-fraction (HF) organic material. The stable isotopes  $^{13}C$  and  $^{15}N$  of the LF and HF were analyzed for their  $^{13}C$  and  $^{15}N$  stable isotope values. Complete understory vegetation removal significantly enriched soil  $\delta^{15}N$  on DFO sites at age 5. The presence of understory vegetation had significant effects on organic matter decomposition and soil nutrient cycling.

**Keywords:** Stable isotopes, Carbon, Nitrogen, Soil organic matter, Vegetation removal

**Öz**

Kesim ve diri-örtü kontrolü orman ekosistemlerinin ekolojik bütünlüğünü tahrip edebilmektedir. Meşçere bitki kompozisyonundaki ani değişiklik sahanın besin durumunu da değiştirmektedir. Bu çalışmanın amacı  $\delta^{13}C$  ve  $\delta^{15}N$  durağan izotoplarından yararlanarak orman ekosisteminde diri örtü kontrolünün besin döngüsüne etkisini belirlemektir. Çalışmada ABD'nin Oregon eyaletinin Pasifik kıyısında bulunan Duglas göknarı ağaçlandırma sahalarında daha önce gerçekleştirilen farklı yoğunlukta diri örtü kontrol çalışmasından yararlanılmıştır. İşlemler farklı yoğunlukta çalı ve otsu türlerin sahadan uzaklaştırılmasını içermektedir. Kontrol ünitesinde diri örtü olduğu gibi bırakılmıştır (DFC). Bir deneme ünitesinde, çalı türleri uzaklaştırılmış ve sahada Duglas göknarı ağaçları ile otsu türler bırakılmıştır (DFH). Bir başka deneme ünitesinde, bütün diri örtü uzaklaştırılmış ve sadece Duglas göknarı fidanları bırakılmıştır (DFO). Ağaçlandırmanın 5. ve 15. yılında her deneme ünitesinden toprak örneklemeleri yapılmıştır. Toprak örneklerinden alt örneklemeler alınarak içerisindeki organik maddenin hafif (LF) ve ağır (HF) fraksiyonlarına ayrılması sağlanmıştır. Daha sonra LF ve HF'nin  $^{13}C$  ve  $^{15}N$  durağan izotop içerikleri belirlenmiştir. Denemenin beşinci yılında diri örtünün tamamının uzaklaştırıldığı DFO deneme ünitesinde toprağın  $\delta^{15}N$  bakımından zenginleştiği belirlenmiştir. Diri örtünün sahada bırakılmasının organik madde ayrışmasında ve besin döngüsünde etkili olduğu ortaya çıkmıştır.

**Anahtar kelimeler:** Durağan izotop, Karbon, Azot, Toprak organik maddesi, Diri örtü kontrolü

## 1. Introduction

Clearing unwanted vegetation on plantation sites is a well-established silvicultural intervention in the Pacific Northwest (Knowe et al., 1992). On the other hand, intensive management practices including brush removal following harvesting may disrupt the ecological integrity of these highly productive forest ecosystems. Dramatic changes in stand species composition may change the site's nutritional status (Bormann et al., 1994).

Essential nutrients including N, are primarily stored in organic forms (Stevenson, 1986; Waring and Running, 2007). Other soil nutrients such as P and S are also reserved in soil organic matter at great quantities (Stevenson, 1986; Wild, 1988). Additionally, soil organic matter enriches the soil exchange capacity, and can be important in building up the soil capacity for exchangeable cations including Ca, K and Mg (Brady and Weil, 1999). Vegetation removal can increase deprivation of soil organic matter and nutrients (Bormann and Likens, 1979; Wild, 1988; Mao et al., 1992).

Soils may rapidly be deprived of nutrients following disturbances such as clear-cutting. Excess nutrients in the soil profile after plant and microbial uptake can move below the rooting zone via water movement. Vegetation expeditiously recolonizing disturbed sites minimizes nutrient and soil organic matter losses by rapid nutrient uptake (Marks and Bormann, 1972; Bormann and Likens, 1979; Kimmins, 1996; Waring and Running, 2007). Thus, recolonization of the sites by disturbance-resistant and pioneering species may significantly ameliorate the effects of severe ecological disturbances, such as clear-cutting, during the period of tree reestablishment in which nutrients and key soil organisms are retained. Management practices that clear these pioneering vegetation can lead to nutrient losses, may hamper ecological diversity, and may, possibly affect sustainability (Perry, 1988). The implications for tree growth of retaining or removing the understory hinges on a balance between the understory's detrimental competition effect and its beneficial soil fertility effects.

The question arises as to whether partial or complete removal of some or all understory components enhances or limits nutrient availability for tree growth in new plantations. Although competing species can reduce seedling growth in the early phases of the stand establishment, broadcast understory vegetation control may, in some cases, be ecologically undesirable. In this study, an effort was made to analyze the soil stable isotopes,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  properties for interpreting effects of forest understory vegetation removal on nutrient

cycling at the ecosystem level. Stable isotopes,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  properties for understanding effects of forest understory vegetation removal on nutrient cycling at the ecosystem level.

## 2. Materials and Method

### 2.1. Study sites

The sites selected to reflect vegetation zones and topographic aspect. Two vegetation zones were located: a Sitka spruce (*Picea sitchensis*) zone that stretches along a narrow band parallel to the Pacific Ocean and a western hemlock (*Tsuga heterophylla*) zone situated several kilometers further inland near Waldport, Oregon. A detailed description of the study sites and methods is presented in Yildiz (2000) and (Wagner and Radosevich, 1989).

Soils in these two zones are deep, relatively fertile, and well drained. They are high in soil C and N, but may be low in S (Cromack et al., 1999). Soils have a low bulk density (0.5-0.6 g cm<sup>-3</sup>) and are highly porous (> 20 cm hr<sup>-1</sup> permeability), resulting in a highly permeable profile that supports generally aerobic conditions that result in rapid turnover of organic matter and release of nutrients (Wagner, 1989).

### 2.2. Experimental Treatments

This study utilized a previously large-scale project where the understory vegetation was differentially controlled in Douglas-fir plantations (Wagner, 1989). Biomass and allocation, organic matter and part of soil data at age 5<sup>th</sup> and 15<sup>th</sup> year was previously published (Yildiz, 2000; Yildiz et al., 2011). The experiment used a randomized block design with four blocks. Each site was composed of seven 20 x 20 m plots. Treatments included seven randomly assigned levels of salmonberry (*Rubus spectabilis* Pursh) and herbaceous weed control. In addition to salmonberry, some other shrub species were present in small quantities. These mainly included thimbleberry (*Rubus parviflorus* Nutt.), red elderberry (*Sambucus racemosa* L. var. *arborescens* [T. & Gray]), vine maple (*Acer circinatum* Pursh), cascara (*Rhamnus purshiana* DC.) and red huckleberry (*Vaccinium parvifolium* Smith). The most abundant herbaceous plant species included velvet-grass (*Holcus lanatus* L.), with lesser coverage of swordfern (*Polystichum munitum* [Kaulf] Presl.), pearly everlasting (*Anaphalis margaritacea* [L.] B. & H.) and foxglove (*Digitalis purpurea* L.).

In April 1985, two-year old bare-rooted Douglas-fir seedlings were planted at a 3 x 3 m spacing following hand-clearing of all existing shrubs using a chainsaw in March 1985. A control plot received no vegetation removal following initial establishment with Douglas-

fir seedlings (DFC). On three of the plots, 25, 50 and 75% of the shrubs were cleared in randomly formed two m wide corridors.

Herbaceous vegetation was allowed to colonize these corridors after shrub removal. On one of the plots, shrubs were completely removed, leaving only herbs and Douglas-fir (DFH). On another plot, all shrubs and 50% of herbs were controlled. Finally, broadcast control of shrubs and herbs was included in the array of treatments, leaving only Douglas-fir (DFO). Each treatment was maintained manually through the growing seasons from 1985 to 1989 (Wagner, 1989).

To minimize deer, elk and rodent damage, each experimental site was fenced by a 2.5 m tall, woven wire fence and, after planting, every seedling in all of the experimental plots was enclosed in a cylindrical, open-top chicken-wire cage (Wagner, 1989).

### **2.3. Data Collection and Analysis**

Soil samples were collected from 0-7.5 cm and 7.5-15 cm soil depths at 5 randomly selected locations on each plot, using a double-cylinder sliding-hammer core sampler at 5<sup>th</sup> and 15<sup>th</sup> year of the stand establishment (Blake and Hartge, 1986). Soil samples were separated into coarse- and fine-fractions, using USA Standard Testing Sieves with 2 mm, 4 mm and 6.3 mm openings. Soil for 1989 was air dried and then sieved. Soil for 1999 was moist sieved. For 1999, field moist soils from DFC, DFH and DFO were sub-sampled for separation of light (LF)- and heavy-fraction (HF) organic material. Twenty grams of soil from < 2 mm fractions was dispersed in sodium polytungstate (NPT) solution (1.7 g cm<sup>-3</sup>) over 24 hr. After stirring, the HF was allowed to settle for 48 hr at room temperature, then the solution was aspirated to separate LF using the methods developed by Strickland and Sollins (1987). For each 1989 and 1999, both coarse- and fine-fractions of remaining soil portions were ground into powder with a heavy duty rock grinder.

Stable isotopes <sup>13</sup>C and <sup>15</sup>N of the LF and HF were analyzed for their <sup>13</sup>C and <sup>15</sup>N stable isotope values using a continuous flow method and a Finnigan Delta Isotope Ratio Mass Spectrometer with ± 0.2% 0 sensitivity for both C and N.

### **2.4. Statistical Analysis**

In order to determine treatment effects on soil density fractionation, <sup>13</sup>C values were analyzed for both LF and HF. Then, differences between LF-HF were analyzed with an analysis of variance procedure for a randomized block design. Repeated measurement analysis with a mixed model was run using data from 1989 and 1999 to determine the

changes in treatment effects over this time period. SAS was used for all statistical analyses (SAS 1996). Results were considered significant at  $P < 0.1$ .

### 3. Results and Discussions

Stable isotope technique is an effective tool to illustrate soil organic matter turnover rates in different ecosystems around the world (Bernoux et al., 1998; Staples et al., 2001; Currie et al., 2004). In the current study, total understory vegetation removal significantly enriched soil  $\delta^{15}\text{N}$  on DFO sites ( $P < 0.09$ ) at age 5 yr. Soil on the DFO plots had the greatest  $\delta^{15}\text{N}$  figures (about 1 per mil higher than in the DFC stand soil) (Table 1).  $\delta^{13}\text{C}$  signatures of  $< 2$  mm soil light (LF) and heavy fractions (HF) were not significantly different between plots, but the differences between LF and HF were significantly affected by understory removal ( $P < 0.04$ ).

**Table 1.** Means (‰) and SE of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in  $< 2$  mm light, heavy, and total soil fractions on Douglas-fir plots at plantation age 5 yr and 15 yr.

Year	Variable	No removal	100% shrub removal	Pure Douglas-fir
1989	Total soil $\delta^{13}\text{C}$	-26.1 (0.10) a	-26.3 (0.07) a	-26.2 (0.12) a
	Total soil $\delta^{15}\text{N}$	1.54 (0.27) a	1.83 (0.12) ab	2.31 (0.14) b
1999	Total soil $\delta^{13}\text{C}$	-26.2 (0.10) a	-26.5 (0.10) a	-26.3 (0.20) a
	Total soil $\delta^{15}\text{N}$	1.78 (0.21) a	1.75 (0.15) a	2.31 (0.22) a
	Light fraction $\delta^{13}\text{C}$	-25.8 (0.26) a	-26.65 (0.26) a	-26.4 (0.26) a
	Heavy fraction $\delta^{13}\text{C}$	-25.7 (0.26) a	-26.1 (0.26) a	-25.8 (0.26) a
	LF-HF $\delta^{13}\text{C}$	-0.09 (0.29) a	-0.57 (0.31) ab	-0.61 (0.44) b

**Note:** Within a row, means with a common lowercase letter are not significantly different at  $P = 0.10$ .

Herb and shrub substrate quality could contribute to rapid composition and nutrient turnover, both above and belowground. Yildiz et al. (2011) reported that the root biomass in the upper 15 cm of soil averaged nearly 10 times greater on the DFC sites than that on the DFO plots at age 5 yr. On the other hand, with relatively higher lignin, the needle dominated forest floor may retard the decomposition rate. Yildiz (2000) stated that the forest floor on the DFO stands averaged 2.5 times greater in biomass than that on DFC sites at age 15 yr. Since turnover of different soil organic matter components varies due to complex interactions of biological, chemical, and physical processes in soil, further investigation is needed to determine the microorganisms dominating organic matter decomposition on different sites. The data imply that organic matter on the DFC sites demonstrated greater decomposition rate at age 5 yr due to the fact that these sites were dominated by shrubs and

herbs. In contrast, the forest floor on the DFO sites manifested slower decomposition due to the more recalcitrant properties of needles. Residual lignin is the C fraction most likely to be preserved during organic matter (humus) decay, since lignin decomposes relatively slowly in the early phases of litter decay (Nadelhoffer and Fry, 1988; Tiunov, 2007). Yildiz (2000) reported that soil C was greater on the DFC sites at this early phase of stand establishment. At age 15 yr, there was no soil C differences among the sites (Yildiz et al., 2011). This may be due to fact that following well-decomposition of leaf litter, lignin and other residual fractions decompose at similar rates. Therefore, during the later stages of litter decay, lignin has also begun decomposing (Nadelhoffer and Fry, 1988). This then contributed to similar soil C content among the sites. To test this hypothesis, we investigated the isotopic composition of the < 2 mm soil fraction. The light fraction (LF) is taken to be less decomposed plant and animal residues with a relatively high C:N ratio and rapid turnover. The heavy fraction is considered to be organomineral complexed soil organic matter which is taken to have comparatively more advanced or humified decomposition products, with a narrower C:N ratio, slower turnover rate, and a higher specific density due to its intimate association with soil minerals (Sollins et al., 1984; Christensen, 1992). Light fraction soil organic matter is likely to be affected by vegetation type, litter production and decomposition. Plant materials have lower  $^{13}\text{C}$  than the soil. Plant litter and roots are both depleted in  $^{13}\text{C}$  relative to the soil (Sollins et al., 1984; Christensen, 1992). As  $^{13}\text{C}$  depleted inorganic C is released through decomposition to the soil solution and to the atmosphere, organic matter particles gradually decrease in size and in C:N ratio and become relatively enriched in  $^{13}\text{C}$  (Nadelhoffer and Fry, 1988; Agren et al., 1996; Wu et al., 2018). Thus, more highly decomposed organic matter is relatively enriched in  $^{13}\text{C}$  compared to new litter inputs. In our study, the light density fraction of the fine soil (< 2 mm) ranged from 5 – 7.5% of soil mass across all treatments. However, the DFO soil was relatively more depleted in  $^{13}\text{C}$  than the DFC soil, possibly due to discrimination against  $^{13}\text{C}$  during rapid plant material decomposition on the DFC sites, bringing on  $^{13}\text{C}$  enrichment in the residual organic matter. Continuous replacement of needle driven organic matter, which is relatively higher in the lignin, may also lower the soil  $^{13}\text{C}$  on the DFO sites, since undecomposed lignin has an isotopic composition similar to fresh plant material (Nadelhoffer and Fry, 1988).

Soil organic matter (SOM) has a pivotal role for the soil fertility (Wild, 1988; Tiessen et al., 1994). Cation exchange capacity of the soil is influenced by humus content (Wild, 1988). Highly mobile ions that are poorly buffered can be lost through disturbance

(Ehleringer et al., 1986). Soil pH is one of the primary constituents in determining soil nutrient availability. High concentrations of  $H^+$  ions may enhance the loss of base cations. Higher amounts of nitrate leaching off the soil may demonstrate : 1) a limitation of energy source (C) for soil microorganisms, 2) increased acidity due to production of  $H^+$  ions during nitrification, and 3) higher removal of cations from the upper soil horizon via nitrate leaching due to negative electrical charges and a high diffusion coefficient of this nutrient in soil (Paul and Clark, 1996).

Soil on the DFO plots had 5-fold greater nitrate than DFC soil at age 5 yr (Yildiz et al., 2011). When the substrate  $NH_4^+$  pool is too large to be consumed by nitrifiers, then  $NO_3^-$  becomes depleted in  $^{15}N$  values (Nadelhoffer and Fry, 1988; Krull et al., 2002). Since nitrification discriminates against  $^{15}N$  in the substrate ( $NH_4^+$ ) more than mineralization does (Hogberg, 1997), the loss of  $^{15}N$  depleted N (leaching  $NO_3^-$ ) would result in  $^{15}N$  enrichment on the DFO plots. Our data showed that  $\delta^{15}N$  values of soil varied among the sites ( $P < 0.09$ ), with the highest values being determined on the DFO sites at age 5 yr. This would help to justify the lower exchangeable Ca on the DFO plots. Data collected 10 years later (1999) did not demonstrate the same trend in soil N isotopic composition, possibly due to greater nutrient uptake and less nitrate leaching due to a more extended nutrient depletion zone by growing Douglas-fir roots.

#### **4. Conclusions**

Isotopic composition of light and heavy soil fractions suggests that organic matter decomposition may vary between sites. Total understory vegetation removal reduced the soil C. The presence of understory vegetation had significant effects on organic matter decomposition and soil nutrient cycling. Continuous replacement of needle driven organic matter, with relatively higher lignin concentrations, may decrease the decomposition rate in pure stands. Further research is needed to investigate changes in soil C and nutrients in the future with the presence and absence of understory as part of ecosystem.

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