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Nodule Phenology and Nitrogen Mineralization of Rhizosphere in Autumn-olive (*Elaeagnus umbellata*) Stand

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보리수나무 군락의 근류계절학 및 근계의 질소무기화

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ABSTRACT

Nodulation phenology in relation to plant phenology, vertical distribution of nodule and root biomass in different soil, correlation between nodule and root size, and nitrogen mineralization around the rhizosphere by ion-exchange resin bag buried at 10 cm of soil were studied in *Elaeagnus umbellata* (autumn-olive) stand, Korea. Nodulation appeared from spring to autumn and nodule phenology was coincided with the timing of root activity rather than that of foliation. Nodule size increased in proportion to the root size. In the sand dune with the lower root biomass, nodule appeared up to 80 cm deep in soil and the nodule biomass was 1,070 kg/ha, which was the highest value reported for several actinorhizal plants in the temperate regions. It is suggested that nodule distribution and production are mainly influenced by soil aeration among environmental factors. The higher ammonification or lower nitrification rate contrasted markedly with the earlier studies that reported lower ammonification or higher nitrification in actinorhizal plant soil. Nitrogen mineralization rate around the rhizosphere with root and nodule was characterized by higher nitrification rate than that in the control soil without root and nodule.

Key words: *Elaeagnus umbellata*, Ion-exchange resin bag, Nitrogen mineralization, Nodule phenology

INTRODUCTION

Elaeagnus umbellata (autumn-olive), is an actinorhizal dinitrogen-fixing shrub, commonly

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found in disturbed areas, alluvium, and poor soil such as sand dune, moraines, road cuts, eroded or air polluted areas (Torrey 1978, You *et al.* 1994a). Because *E. umbellata* improves soil fertility and facilitates the growth of interplanted species, it has been widely utilized as nurse plant for economic crops, for restoration of disturbed areas or as ornamentals in urban plantings (Uemura 1971, Friedrich and Dawson 1984, Paschke *et al.* 1989, Wheeler and Miller 1990).

Several studies report the geographical distribution, population dynamics and factors affecting nitrogen fixation of autumn-olive (Song *et al.* 1993, 1994, You *et al.* 1994a, 1994b), but there is only a little information available on the interaction of nodule dynamics with plant phenology or seasonal pattern of nitrogen mineralization around the rhizosphere in the field.

In this study, we investigated nodulation in relation to plant phenology, vertical biomass distribution of nodule and root, the relationship between nodule size and root size, and nitrogen mineralization around the rhizosphere in *E. umbellata* stand, Korea.

MATERIALS AND METHODS

Phenology of *E. umbellata*

Phenology including foliation and leaf yellowing, flowering, flower aging, fruit developing, fruit maturing, fine rooting, and nodulation of autumn-olive was observed biweekly during the ice-free season in Namhansansung for 7 representative trees in 1992. Nodule initiation was examined by the light microscope from sampled nodules on root.

Vertical distribution of root and nodule biomass

Three quadrats (30cm×30cm) were set up around 30 cm apart from a standard stem of autumn-olive and sampled soil from top soil to 100 cm soil depth at 10 cm interval. The sampled soil was sieved into plant parts and soil. The plant parts were washed with tap water and divided into large roots ($\phi \geqq 2$mm), fine roots ($\phi < 2$mm) and nodule. Root and nodule diameter was measured with vernier calipers. These samples were dried at 80℃ for 74 hrs and weighed.

We selected three study sites with different soil substrates such as sand dune, granite and limestone. The sand dune site was located at Daenanjido (37°00′N, 126°25′E), Sungmun-myun, Dangjin-gun, Chungchungnam-do, the granite site at Namhansansung Provincial Park (37°28′N, 127°11′E), Jungbu-myun, Kwangju-gun, Kyunggi-do, and the limestone site at Kakkiri (37°01′N, 128°17′E), Juksung-myun, Danyang-gun, Chungchungbuk-do, Korea. The density of autumn-olive and the stand age were 2,100 trees/ha, 19 years for sand dune, 457 trees/ha and 19 years for granite, and 714 trees/ha and 18 years for limestone soil, respectively. All sites have a typical temperate climate with heavy rain and high temperature in summer and relatively little rain and low temperature in winter. The sand dune was affected by seawind and salt spray. Additional information such as general climate condition and soil physico-chemical data on these study sites was shown in the previous reports (You *et al.* 1994a, 1994b).

Nitrogen mineralization rate around rhizosphere

Ion-exchange resin (IER) bags were used to quantify nitrogen mineralizaion and nitroge release from the rhizosphere (Binkley and Matson 1983). IER bags were constructed by sealing 15 g of oven dry weight of mixed-bed cation + anion resin in nylon nets (mesh size = 0.25 mm). −Diaion SA 20A and + Diaion SK1B were used for nitrate and ammonium absorption, and were charged with 0.5 N $NaHCO_3$, 0.5 N HCl for 30 min, respectively.

The nitrogen mineralization rate from the rhizosphere with root and nodule was measured at control site only without root under *E. umbellata* inner marginal canopy (You *et al.* 1994b) and at the rhizophere site with root nodule within the crown area in Namhansansung. Thus in this control site, when compared with the rhizosphere site, only the aboveground leaf and branch litter among plant factors may affect nitrogen mineralization.

Resin bags (n=7) were buried monthly in soil at 10cm depth, where nodule mainly appeared, as follows; the entire top layer soil blocks of 20cm × 20cm to a depth of 10cm were transported by shovel and then the spreaded bags were left and covered with the moved soil blocks, carefully. All sites are plain.

IER bags sampled monthly from March to December 1993 were immediately brought to the laboratory, where the bags were extracted with 100 ml of 2 N KCl for 30 min. Samples were equlibrated overnight at 4℃ and then filtered (Whatman No. 42 filter paper) and stored at −4℃ until analysis. Nitrate and ammonium nitrogen were determined by cadmium reduction and phenate method (APHA 1989), respectively.

Statistical analysis

The relationship between nodule and plant root size was analyzed by PROC CORR and the difference of nitrogen mineralization among sites by PROC TTEST subprograms of SAS (Sas institute 1979).

RESULTS

Phenology of *E. umbellata*

Belowground activity such as nodulation and fine rooting started prior to aboveground foliation (Fig. 1). Fine rooting appeared in two times in early spring and autumn with a gap in summer, and these phenomena are commonly observed in the trees in temperate regions (Santantonio and Herman 1985). Nodulation continued from spring to autumn except winter, and occurred on the large root during the period (early June to late-August) without fine rooting. Nodule growth of autumn-olive started earlier than *Myrica gale*, winter-deciduous actinorhizal species (early May; Schwintzer *et al.* 1982). Root initiation can

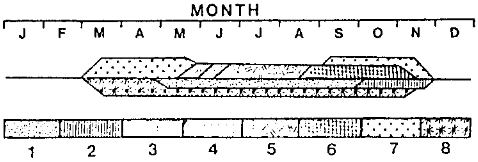

Fig. 1. Phenological spectrum for *E. umbellata* in Namhansansung, Korea (1, foliation; 2, leaf yellowing; 3, flowering; 4, flower aging; 5, fruit developing; 6, fruit maturing; 7, fine rooting; 8, nodulation).

occur at very low temperatures, with *Eriophorum angustifolium* having optimum root initiation at 5℃ (Chapin 1974).

Vertical distribution of root and nodule biomass

There were marked differences in vertical distribution of belowground biomass between the sand dune and the other sites (Fig. 2). In sand dune, biomass of root and nodule was distributed at the deeper soil than that in granite and limestone. The vertical distribution pattern of nodule biomass was similar in that of the root, but the correlation between nodule biomass and small and large root biomass was insignificant ($r = 0.05$, $P > 0.5$). Nodule biomass was distributed in the upper 0~30 cm of soil (100%) at granite and limestone soil, but in the deeper 31~60cm soil (75%) at sand dune.

Total Biomass of belowground part was the largest in granite, the smallest in limestone soil with higher plant density (Table 1). Total root biomass including fine and large root

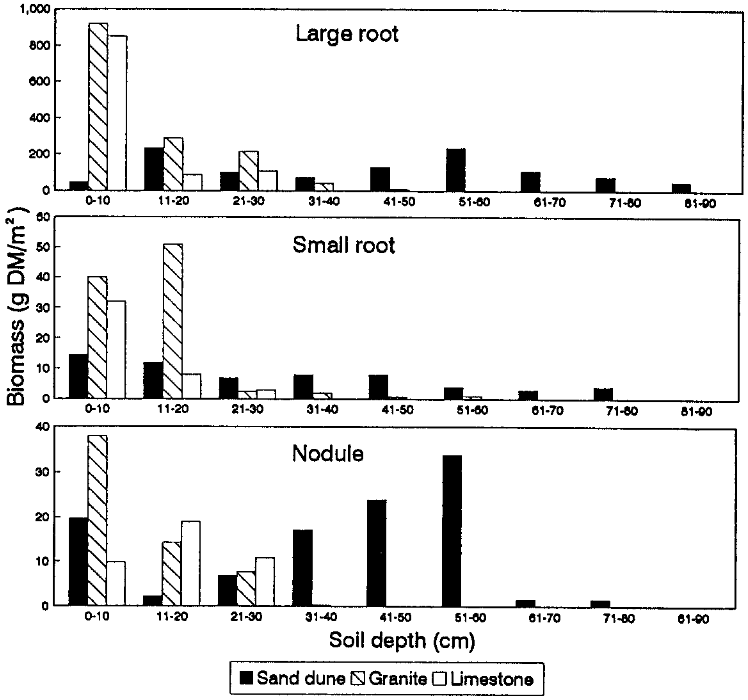

Fig. 2. Vertical distribution of biomass of large root (upper, $\phi \geqq 2$mm), fine root (middle, $\phi < 2$mm) and nodule (lower) of *E. umbellata* in sand dune, granite and limestone.

Table 1. Belowground standing biomass of large root ($\phi \geqq 2$mm), fine root ($\phi < 2$mm) and nodule of *E. umbellata* community in sand dune (Dananjido), granite (Namhansansung) and limestone (Kakkiri) area. Value in parenthesis is percentage to total in each site

Site	Biomass (g DM /m^2)			
	Large root	Fine root	Nodule	Total
Sand dune	1,053 (86.3%)	60 (5.0%)	107 (8.8%)	1,220 (100%)
Granite	1,473 (90.3%)	97 (5.9%)	60 (3.7%)	1,631 (100%)
Limestone	1,048 (92.7%)	43 (3.8%)	40 (3.5%)	1,131 (100%)

in sand dune (1,139g DM /m^2) was less than that in granite (1,570g DM /m^2), but the nodule biomass showed the opposite pattern to the root (107:60g DM /m^2) between the two sites. The ratio of nodule biomass to total belowground parts in sand dune was about twice as much as that in the others.

E. umbellata nodules are much-branched coralloid type that range from < 0.1cm to 5.3cm in diameter. Nodule diameter was significantly related to the large root size ($Y = 0.86x + 6.81$; $r = 0.62$, $P < 0.001$). This result indicates that *E. umbellata* nodule is perennial.

Nitrogen mineralization rate around rhizosphere

Monthly ammonification rate was higher than nitrification rate during all months in control (mean 7.37:0.28kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) and in the rhizosphere site (6.45:0.29 kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) (Fig. 3a). Ammonification rate (mean±SD) was lower in rhizosphere (9.66±4.75kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) than in control (11.29±5.02kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}), especially in April ($t = 2.53$, $P<0.05$), July ($t = 3.68$, $P < 0.05$) and September ($t = 5.78$, $P < 0.05$). Ammonification rate was highest in July (17.72kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) in control, and in June (15.83kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) in rhizosphere site.

On the contrary, the seasonal variation of ammonification rate, monthly nitrification rate, mothly nitrification rate in the rhizosphere site (0.95±1.37kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) was higher than that in control (0.42±0.75kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}), especially in June ($t = 2.52$, $P < 0.05$) and July ($t = 2.68$, $P < 0.01$). The maximum rate of nitrification, recorded in August, was 2.37kg N $\cdot$ ha^{-1} $\cdot$ month^{-1} in control, and 4.43kg N $\cdot$ ha^{-1} $\cdot$ month^{-1} in rhizosphere (Fig. 3b).

Monthly variation of total nitrogen mineralization rate ($NH_4^+ + NO_3^-$) in the rhizosphere (10.60±5.70kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) was nearly equaled to that in control (11.70±5.40kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}), except in April (14.00:9.00kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}, $t = 2.21$, $P < 0.05$). The highest mineralization rate was occurred in August in control (18.35kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) and in rhizosphere (18.72kg N $\cdot$ ha^{-1} $\cdot$ month^{-1}) (Fig. 3c).

The annual rate of ammonification was much higher than that of nitrification at both sites (Table 2). The amount of nitrification over ammonification was small in both sites, but the contribution percentage to total was higher in rhizosphere site (9%) than in control (4%). This result means that nitrogen mineralization of belowground part including root and nodule was characterized by higher nitrification than that of aboveground parts

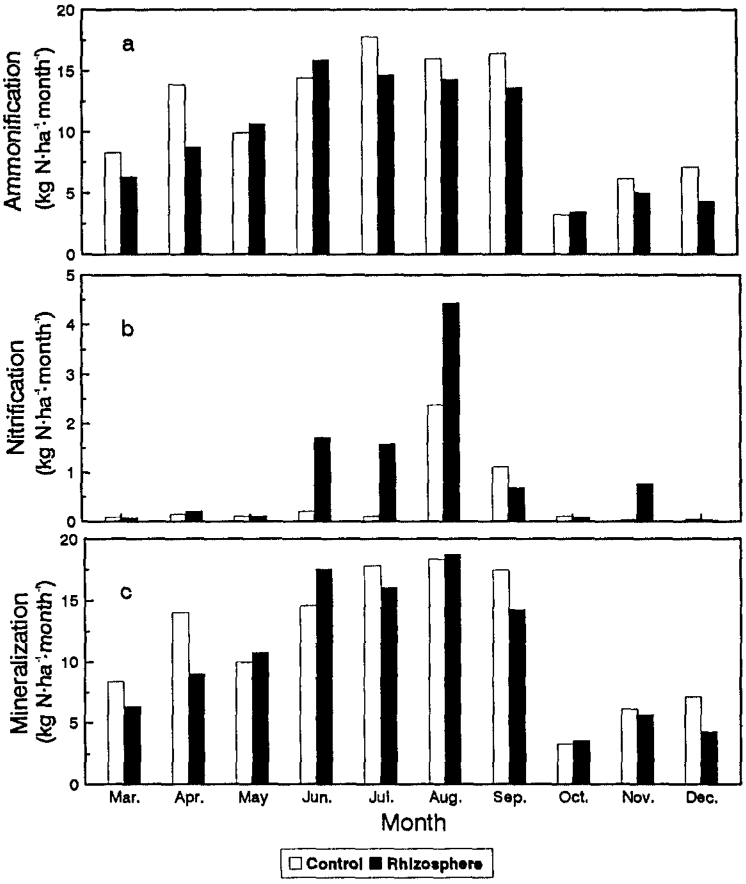

Fig. 3. Seasonal nitrogen release by ammonification (NH_4^+) (a), nitrification (NO_3^-) (b) and mineralization ($NO_3^- + NH_4^+$) (c) by ion-exchange resin bags in the 10cm of soil in control and rhizosphere site during April to December 1993 in *E. umbellata* stand.

such as leaf and branch litter in *E. umbellata* community.

Table 2. Total nitrogen mineralization rate (kg N · ha^{-1} · 9 $month^{-1}$) by ion-exchange resin bags buried at 10cm of soil from April to December 1993 in *E. umbellata* stand. Value in parenthesis is percentage to sum

Mineralization form	Site	
	Control	Rhizosphere
Ammonification	113 (96%)	97 (91%)
Nitrification	4 (4%)	10 (9%)
Sum	117 (100%)	106 (100%)

DISCUSSION

Nodulation and fine rooting started prior to or posterior to foliation (Fig. 1) and it means that energy source used for belowground plant activity and nodule formation comes from photosynthetic substance saved in the previous growing season (Wheeler *et al.* 1983). Nodule growth timing of *E. umbellta* was correlated with the root rather than the aboveground activity such as budbreak (Schwintzer *et al.* 1982). Considering the report that the acetylene reduction ability of autumn-olive began with the shoot growth (April, Song *et al.* 1994), it is estimated that it takes one month for nitrogen fixation after nodule initiation. In summer, fine rooting disappeared, but nodulation appeared on the large root. This fact indicates that *Frankia* infects through cortical cells in *E. umbellata* (Kim *et al.* 1993).

Belowground biomass was distributed relatively deep in sand dune (Daenanjido), when compared with that in other soil where most nodule in actinorhizal plant is located mainly on top or subtop soil layer (Grove and Malajczuk 1992, Song *et al.* 1994) as in granite and limestone soils in this study (Fig. 2). Nodule standing biomass of 1,070kg /ha in sand dune in this study with lower root biomass was higher than any previously reported maximum value of 750kg /ha for actinorhizal plants in the temperate regions (Mcnabb and Cromack 1982). The sand dune was consisted of only silt in soil texture and has a poorer nutrient and water condition than the granite or the limestone soil (You *et al.* 1994a). Thus this higher nodule biomass in sand dune suggests that soil aeration among soil factors affecting nodule distribution and production be the most important one (Song *et al.* 1994).

The correlation between nodule biomass and small or large root biomass was not significant, but positive relationship between nodule diameter and root diameter was existed. This relationship may result from the fact that the nodule regenerates randomly with a time lapse because nodule of *E. umbellata* is perennial.

Ammonium nitrogen was higher than nitrate nitrogen at both sites throughout the experimental period (Fig. 3a-b). The dominance of ammonification in *Elaeagnus* soil was incongruous to the similar studies in actinorhizal plant plot or actinorhizal interplanting site. Rietveld *et al.* (1983) and Paschke *et al.* (1989) found that in the actinorhizal nitrogen fixer stands, NO_3^--N was the major form of mineral nitrogen available, but in the control stands without root and nodule NH_4^+-N was the major, and the same phenomenom was observed in non-actinorhizal deciduous forest, too (Pastor *et al.* 1984). This lower nitrification or higher ammonification rate pattern means that nitrogen immobilization controls at the level of ammonification in this actinorhizal plant community soil. The inhibition of nitrification in this community can be explained by the fact that soil pH 5.2~5.5 in this study area (You *et al.* 1994a) is more acidic than optimal pH 6.6~6.8 (Paschke *et al.* 1989), and that the soil temperature of this site estimated from air temperature 27℃ (You *et al.* 1994a) is probably lower than optimal temperature 30~35℃ for nitrification, or that self-allelochemicals may be produced from *Elaeagnus* plants (White 1988). In other studies, available phosphate content in soil (Pastor *et al.* 1984), the number of nitrifying bacteria (Vitousek *et al.* 1982) and nitrogen concentration of nodule (Wardle and Greenfield 1991) were also found to be the major factors for regulating nitrogen mineralization in soil.

Estimated annual production of mineral nitrogen in this soil was less than that in walnut plot with *Elaeagnus* (236kg N · ha^{-1} · yr^{-1}) or *Alnus* (185kg N · ha^{-1} · yr^{-1} (Paschke *et al.* 1989), but this value was more than that in some deciduous non-actinorhizal forest soil types (53~84kg N · ha^{-1} · yr^{-1}, Pastor *et al.* 1984; 40~56kg N · ha^{-1} · yr^{-1}, Binkley and

Walentine 1991).

Atmospheric inorganic nitrogen inputs (14.9kg $N \cdot ha^{-1} \cdot yr^{-1}$ for ammonium, 5.23kg $N \cdot ha^{-1} \cdot yr^{-1}$ for nitrate) via precipitation during our study period in Mt. Kwanak, which is located about 10 km eastward from our study site (Kim 1994), represented 13~15% for ammonium, and 55~100% for nitrate, respectively. When corrected by the deposition input, there is no net nitrification in control site. Thus the belowground nitrogen mineralization was characterized by higher nitrification rate in *E. umbellta* community.

In April, interestingly, both ammonification rate and total mineralization rate were lower in the rhizosphere than those in control site (Fig. 3a-c). This result might be due to the fact that plants absorb inorganic nitrogen from the soil during their foliation activity (Fig. 1).

적 요

보리수나무의 근류계절학, 토양이 다른 곳에서 생육하는 근류와 뿌리 건물량의 수직분포, 근류크기와 뿌리간의 관계, 토심 10cm에서 resin주머니법에 의한 근권의 질소무기화를 야외에서 연구하였다. 근류는 겨울을 제외하고 연중 형성되었고, 뿌리의 활동시기와 일치하였다. 근류크기는 뿌리의 직경에 비례하여 증가하였다. 사구에서 근류는 토심 80cm까지 깊게 분포하였고, 그 뿌리의 무게는 적었으나 근류건량은 1,070kg/ha로 온대지방에서 보고된 값 중 가장 많았다. 이러한 사구의 연구결과는 토양의 통기가 보리수나무의 토양 내 수직분포와 근류생산에 중요한 요인임을 의미한다. 보리수나무군락 토양의 질소무기화는 다른 비콩과 질소고정식물의 것보다 암모니아화는 많이, 질산화는 적게 나타났다. 군락내에서 뿌리와 근류가 있는 근권에서 일어나는 질소무기화는 그것이 없는 대조구에 비하여 질산화율이 높은 것이 특징이었다.

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