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Seasonal changes in redox properties of peat, nutrition and phenology of *Menyanthes trifoliata* L. in a floating peat mat in Mizorogaike Pond, central Japan

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Abstract

Seasonal changes in leaf population per area, nutrient absorption rate and elemental concentration of *Menyanthes trifoliata* L. were studied in a floating peat mat in Mizorogaike Pond, central Japan, with reference to the peat redox potential. Leaf population of *M. trifoliata* showed a rapid increase in May, reached the maximum in June, and then decreased to 12% of the maximum density in July. The foliage density of *M. trifoliata* again increased and reached its second maximum in September, and then it decreased to zero in December. The decrease of the foliage density from June to July corresponded to the minimum of redox potential (Eh) of the surface peat. Low Eh in the rhizosphere of *M. trifoliata* relates to the temporary disappearance of the foliage of the plant from June to July. Nutrient concentration (K, Mg, Ca, Fe) in *M. trifoliata* roots showed minimum in June to August. The nutrient absorption rate of *M. trifoliata* evaluated from the rubidium absorption rate of excised root of *M. trifoliata* showed a minimum value in June and July. Peat redox properties would affect the nutrient absorption activity of the roots and the consequent foliage phenology of *M. trifoliata* in Mizorogaike Pond.

Abbreviations: EC – electrical conductivity; Eh – redox potential; NHE – normal (standard) hydrogen reference electrode

Introduction

Temporary defoliation during plant growing seasons is known to some wetland species such as *Scilla scilloides* Duruc or *Alnus japonica* (Thunb.) Stend (Kikuzawa 1991). Defoliation during the growing season is discussed following dry matter economy of the plant species (Kikuzawa 1991). Dry matter in leaves can be used for the formation of new organs such as reproductive parts or new leaves. In the case of *A. japonica*, reproduction of new high-quality leaves results in a higher dry matter production rather than elongating the life of the old low-quality leaves. Hence, the leaves of *A. japonica* temporarily disappear in the summer season during the displacement of the old by new leaves. Foliage of *Menyanthes trifoliata* L. in Mizorogaike Pond also shows temporary disappearance in mid summer (Haraguchi 1993). Two to three weeks of leaflessness could be a disadvantageous phenological property for the dry matter production of *M. trifoliata*. So, why does this species lose its leaves for as long as 2-3 weeks in the middle of the growing season?

In wetland communities, the inorganic soil environment is one of the important factors determining the distribution and performance of aquatic plants. In communities on temporarily waterlogged soil or sediment, water table conditions and the consequent aeration of soil determine the distribution and growth of plants (Waughman 1980; Yamasaki and Tange 1981; Armstrong et al. 1985; Yabe 1985; Howes et al. 1986).

Among the inorganic environments which affect the distribution and growth of plants in waterlogged sites, redox potential (Eh) is regarded as an indicator for evaluating the effects of oxygen conditions in the rhizosphere on plant growth. Some studies on salt marshes suggest that Eh is the most important factor for controlling the growth and distribution of plant species (Howes et al. 1986).

A previous study on the redox properties of peat in Mizorogaike Pond showed that the peat surface was temporarily highly anoxic just after the peat surface emergence, following the floating up of the peat mat in summer (Haraguchi 1991a; Haraguchi 1995). So, highly anoxic peat condition in the high-temperature season could be one of the determinant factors of the phenological property of *M. trifoliata* in Mizorogaike Pond. And I investigated the relationship between the Eh of peat and the phenology of *M. trifoliata* in Mizorogaike Pond with reference to nutrient absorption by plants. By monitoring the seasonal changes in leaf population per area, nutrient absorption rate and elemental concentrations (Na, K, Mg, Ca, Fe, Mn) of *M. trifoliata* together with the determination of Eh of peat, I tested the hypothesis that the anoxic soil environment should cause the temporal disappearance of leaves of *M. trifoliata* in Mizorogaike Pond.

Materials and methods

Study area and plant material

The study site was Mizorogaike Pond (c. 400 m × 200 m, maximum depth c. 2 m), situated north of Kyoto City (35° 03′ N, 135° 46′ E, 75 m a.s.l.). An extensive floating mat of peat (c. 240 m × 140 m) covers the pond (Haraguchi 1991a).

Menyanthes trifoliata has a circumboreal distribution around the Arctic Circle (Hewett 1964). *M. trifoliata* distributed rather common in the cool temperate zone in Japan, while in the warm temperate zone of southwestern Japan it shows localized, discontinuous distribution. Mizorogaike pond is one of the areas where *M. trifoliata* grows as a relic species (Kokawa 1961). *M. trifoliata* dominated the population of the mat except for the center of hummocks in the floating mat. The foliage of *M. trifoliata* begins to develop in the middle of March and senesces in mid-December in Mizorogaike Pond (Haraguchi 1993). Inflorescences of *M. trifoliata* appear in September and they remain dormant over winter. They begin to grow in early March and flowering begins in early April. Seeds are produced in late April to May.

Phenology of M. trifoliata

Three quadrats with an area of 1 × 1 m^2 area were selected in the *M. trifoliata* dominated hollow in Mizorogaike Pond. Coverage of *M. trifoliata* was almost 100 % in each of the quadrat. The number of alive leaves of *M. trifoliata* within each quadrat was counted in the field at 7-10 day interval (47 times) from 10 January to 21 December 1989. Relative number of alive leaves of *M. trifoliata* to the maximum number of alive leaves among the three quadrats during the survey period (648 m^{-2}) was used as a parameter for leaf population of *M. trifoliata*. Parts of the data for *M. trifoliata* phenology appeared in Haraguchi (1993).

Measurements of Eh

Redox potential (Eh) at the peat surface was measured in the field at two of the three quadrats which *M. trifoliata* dominated. Averages of 10 measurements at each of the quadrats were used as the representatives of each quadrat. Measurements of Eh were carried out using a potential meter (Horiba HD-7 LD, Horiba Co. Ltd., Kyoto, Japan) equipped with a platinum electrode and an Ag/AgCl (3.33 mol l^{-1} KCl) reference electrode. The electrode was inserted vertically into the peat until 3 cm depth, just among the rhizosphere of the *M. trifoliata*. The depth of the peat from the surface to the rhizosphere was 2-3 cm at all the measurement points. Data are indicated as the potential compared to the NHE (normal (standard) hydrogen reference electrode). Parts of the data for the Eh of peat in Mizorogaike Pond appeared in Haraguchi (1991a) and Haraguchi (1995). Data were collected 24 times from 10 January to 21 December 1989.

Water chemistry

Peat surface water was collected in 250 ml polyethylene bottles at two of the three quadrats (the same as Eh measurements) which *M. trifoliata* dominated, and then carried to the laboratory within a dark box without cooling. Peat was removed by decantation, and then pH and electrical conductivity (EC) of the water were measured within 4 hours after sampling by using potential meter (HD-7LD, Horiba Co. Ltd., Kyoto, Japan) equipped with pH electrode and electrical conductivity meter (DS-7, Horiba Co. Ltd., Kyoto, Japan), respectively. Samplings were made 24 times from 10 January to 21 December 1989 at the same time as Eh measurements.

Plant sample collection

Roots of *M. trifoliata* were sampled from 10 shoots inside the *M. trifoliata* dominated area at ca. 1 m from one of the six quadrats. Roots nearest to the shoot apex were excised in the field. The excised root sample was stored within water and peat collected in 250-ml polyethylene bottles just beside the root sampling point, and carried to the laboratory within dark box without cooling. Samplings were made 24 times from 10 January to 21 December 1989.

Mineral composition of roots

Four of the 10 sampled roots were used for the determination of mineral concentration of the roots. These four samples were flushed three times with distilled water within 4 hours after sampling and dried at 75 °C for 72 hours, then weighed. Acid degradation of dried root followed Rorison and Robinson (1986). Dried root was collected in a crucible, weighed, and then ignited in the electric muffle furnace at 500 °C for 3 hours. After cooling, 5.0 ml of concentrated HCl was added to and heated for 15 min. Then 1.0 ml of concentrated HNO_3 was added, and then heating was continued until the solution dried up. After that, 1.0 ml of 1.0 mol l^{-1} of HCl was added, and then distilled water was added to total 5.0 ml. Sodium, potassium, magnesium, calcium, manganese and iron concentrations were determined once per sample by atomic absorption spectrometer (AA-8200, Nippon Jarrel Ash Co. Ltd., Tokyo, Japan).

Nutrient absorption rate of roots of M. trifoliata

The nutrient absorption rate of roots of *M. trifoliata* was measured following the procedures in Kramer (1969). Six of the 10 sampled roots were used for the measurements of the rate of nutrient absorption. Rubidium was used as tracer of nutrient absorption by the plants (Kramer 1969; Faber et al. 1991). Excised roots were flushed three times with distilled water within 4 hours after sampling, and then soaked in a mixed solution of $1.0\text{x}10^{-2}$ mol l^{-1} RbCl and 5.0×10^{-4} mol l^{-1} $CaCl_2$ for 60 min at 30 °C within 4 hours after sampling. The calcium saturates the cation exchange capacity of the roots, leaving no sites for non-metabolic adsorption of rubidium (Kramer 1969). After the incubation, samples were flushed three times with distilled water, dried at 75 °C for 72 hours, and then weighed. The procedure for the determination of Rb concentration in the roots (once per sample) was the same as that for the root composition determination.

Statistical analysis

The Kruskal-Wallis test was performed on the data for leaf population per area of *M. trifoliata*, Rb absorption rate by excised roots of *M. trifoliata*, chemical parameters of surface peat pore water and components of roots of *M. trifoliata*. Data for each parameter were classified into classes of sampling time (47 classes for leaf population and 24 classes for the other chemical parameters), and differences between classes was measured in order to test the significance of seasonal change for each parameter.

Results

Seasonal change in leaf population of M. trifoliata

Seasonal change in leaf number per shoot of *M. trifoliata* showed two peaks, in May and September (Figure 1), and the same seasonal pattern of foliage phenology was observed every year from 1986 to 1992 in Mizorogaike Pond (Haraguchi 1993; Haraguchi, unpublished data). The leaf population per area increased from 14 March and reached a first maximum on 21 May (516, 476, 648 leaves m^{-2} in each quadrat, respectively). Then the leaf population per area decreased and became 12 % of the maximum on 2 August. Leaves of *M. trifoliata* disappeared during this season in many stands. Re-expansion of leaves occurred and the leaf population increased from 2 August and became 31 % of the maximum number on 3 September. Leaves disappeared again on 5 December and the leafless season continued till the next March. Seasonal change in leaf population of *M. trifoliata* was significant ($p < 0.001$).

Seasonal changes in peat pore water chemistry

The pH of the peat pore water increased from 10 January (4.93) to 28 March (5.24), then decreased to

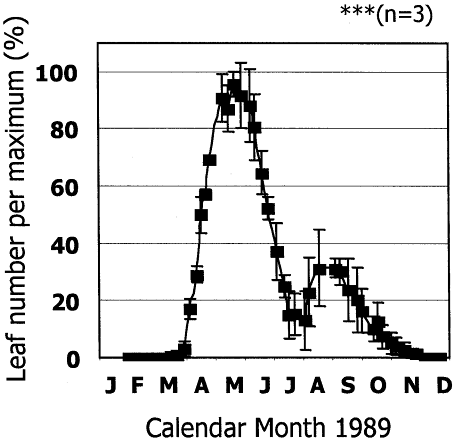

Figure 1. Seasonal change in the leaf population (number per maximum leaf number in three 1x1 m^2 quadrats) of *Menyanthes trifoliata* within the *M. trifoliata* community (n=3) in a floating peat mat in Mizorogaike pond, central Japan. Means and SD (vertical bars) are presented. Parts of the data appear in Haraguchi (1993). n: number of repetitions (quadrats). Significance level for seasonal change tested by the Kruskal-Wallis test, ***: $p < 0.001$

4.88 on 28 May, and then increased to 5.35 on 7 August. After the maximum, pH decreased to 4.64 on 5 December (Figure 2a). Seasonal change in pH of the peat pore water was not significant ($p > 0.05$).

The water EC showed a gradual decrease from 10 January (3.98 mS m^{-1}) to 28 May (2.79 mS m^{-1}), increased to 5.65 mS m^{-1} on 17 August, and then decreased to 2.39 mS m^{-1} on 19 September. After the minimum, EC again increased and showed a maximum of 6.41 mS m^{-1} on 14 November (Figure 2b). Seasonal change in the EC of the peat pore water was significant ($p < 0.05$).

Eh of the peat in two quadrats were not significantly different ($p > 0.05$; n = 10). The averaged Eh of peat surface fluctuated between 117-195 mV from 10 January to 25 April, decreased to a minimum of 10 mV on 23 July, and then increased to 192 mV on 5 December (Figure 2c). This seasonal trend in Eh was observed every year from 1985 to 1992 in Mizorogaike Pond (Haraguchi, 1991a, 1995; Haraguchi, unpublished data). Seasonal change in Eh of the peat pore water was significant ($p < 0.05$).

Seasonal changes in root elemental concentration of M. trifoliata

The Na concentration in roots fluctuated between 3.9-5.7 mg g^{-1} from 10 January to 28 March, decreased to 1.7 mg g^{-1} on 25 April, and then fluctuated between 1.4-2.0 mg g^{-1} until 23 July. After that, Na concentration increased to 5.3 mg g^{-1} on 5 December (Figure 3a). Seasonal change in Na concentration of the root of *M. trifoliata* was significant ($p < 0.001$).

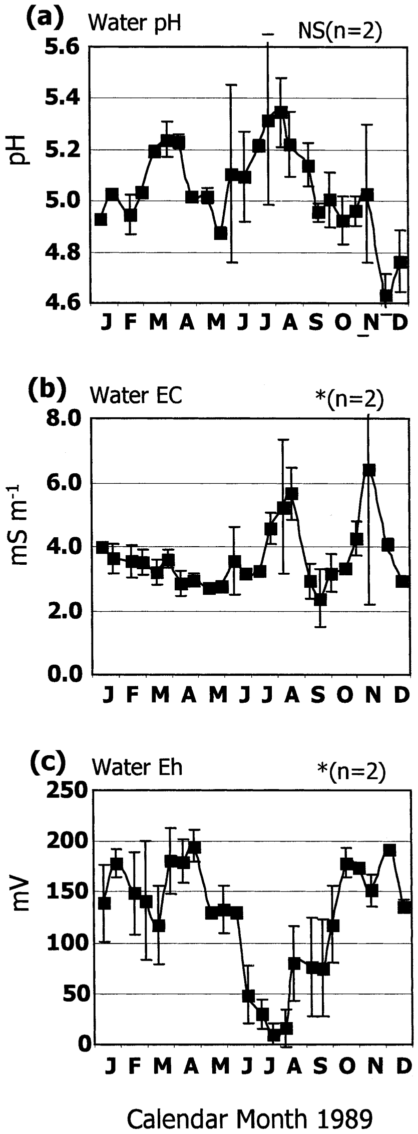

Figure 2. Seasonal changes in (a) pH, (b) electrical conductivity (EC) and (c) redox potential (Eh corrected at pH = 7.0) of the peat pore water at the surface peat in two quadrats (1x1 m^2 ; two quadrats from the three quadrats for leaf population measurement) within the *Menyanthes trifoliata* community in a floating peat mat in Mizorogaike pond, central Japan. Means of the two quadrats and SD (vertical bars) are presented. Parts of the data in (c) appear in Haraguchi (1991) and Haraguchi (1995). n: number of repetitions (quadrats). Significance level for seasonal change tested by the Kruskal-Wallis test, *: $p < 0.05$, NS: not significant $p > 0.05$

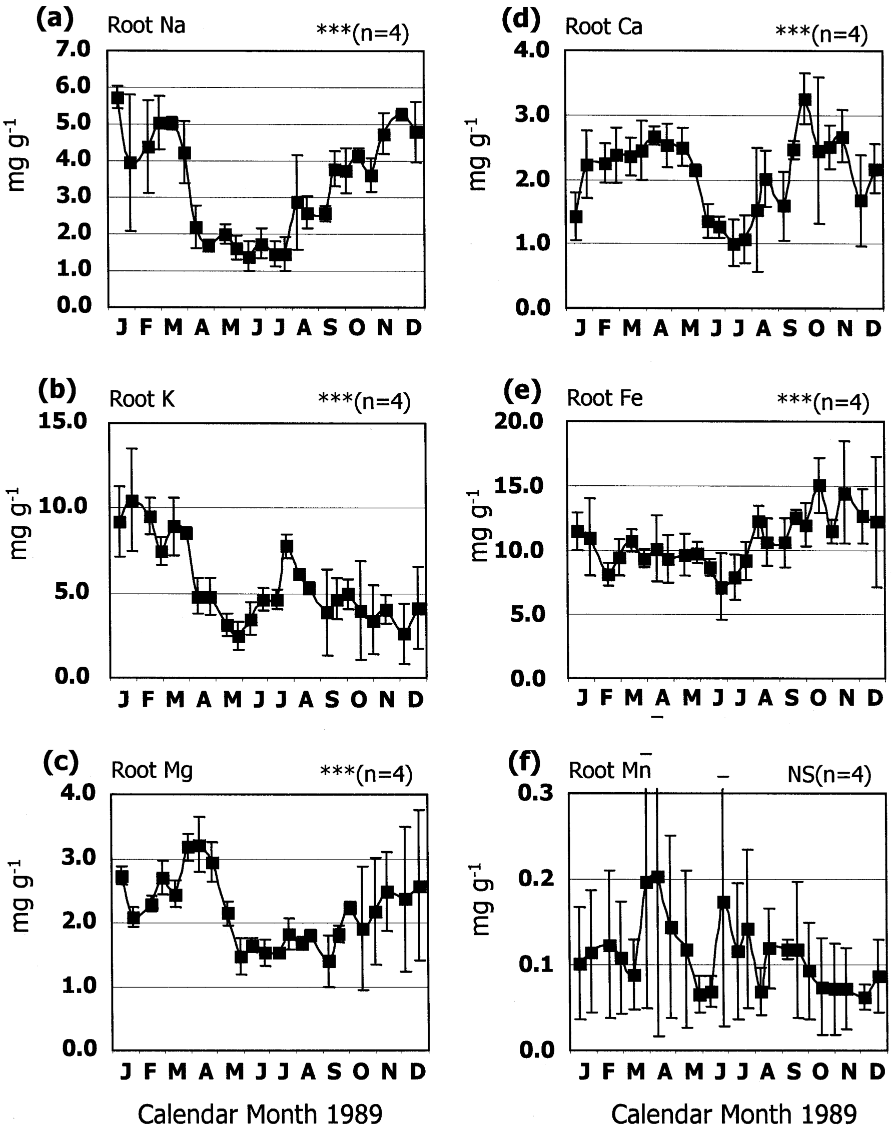

Figure 3. Seasonal changes in (a) Na, (b) K, (c) Mg, (d) Ca, (e) Fe and (f) Mn in the roots of *Menyanthes trifoliata* within the *M. trifoliata* community in a floating peat mat in Mizorogaike pond, central Japan. Root samples were collected near by a quadrat for leaf population measurement (n = 4). Means and SD (vertical bars) are presented. n: number of repetitions. Significance level for seasonal change by the Kruskal-Wallis test, ***: $p < 0.001$, NS: not significant $p > 0.05$

The K concentration in roots decreased to a minimum of 2.5 mg g^{-1} from 10 January (9.2 mg g^{-1}) to 28 May, increased to 7.8 mg g^{-1} on 23 July, and then decreased to 2.6 mg g^{-1} on 5 December (Figure 3b). Seasonal change in K concentration of the root of *M. trifoliata* was significant ($p < 0.001$).

The Mg concentration in roots reached a maximum of 3.2 mg g^{-1} on 11 April, decreased to 1.5 mg g^{-1} on 28 May, and then fluctuated between 1.4-1.8 mg g^{-1} until 19 September. After that, Mg concentration increased to 2.6 mg g^{-1} on 21 December (Figure 3c). Seasonal change in Mg concentration of the roots of *M. trifoliata* was significant ($p < 0.001$).

The Ca concentration in roots fluctuated between 2.2-2.6 mg g^{-1} from 24 January to 14 May, decreased to 1.0 mg g^{-1} on 11 July, and then increased to 3.3 mg g^{-1} on 1 October. After the maximum, Ca concentration decreased to 1.7 mg g^{-1} on 5 December (Figure 3d). Seasonal change in Ca concentration of the root of *M. trifoliata* was significant ($p < 0.001$).

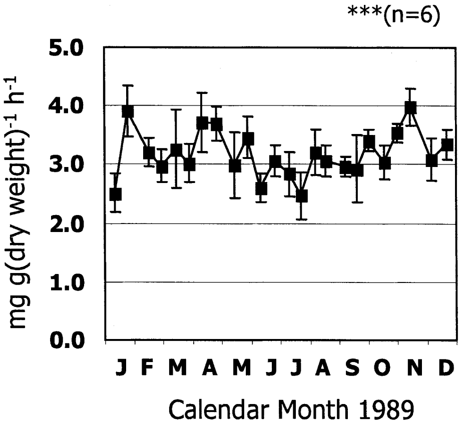

Figure 4. Seasonal change in Rb absorption rate of excised root of *Menyanthes trifoliata* within the *M. trifoliata* community in a floating peat mat in Mizorogaike pond, central Japan. Root samples were collected near by a quadrat for leaf population measurement (n=6). Means and SD (vertical bars) are presented. n: number of repetitions (samples). Significance level for seasonal change tested by the Kruskal-Wallis test, ***: $p < 0.001$

The Fe concentration in roots decreased from 10 January (11.5 mg g^{-1}) to a minimum of 7.1 mg g^{-1} on 25 June, and then increased to 15.0 mg g^{-1} on 17 October (Figure 3e). Seasonal change in Fe concentration of the root of *M. trifoliata* was significant ($p < 0.001$).

The Mn concentration in roots fluctuated between 0.06-0.20 mg g^{-1} and it did not show statistically significant seasonal change ($p > 0.05$; Figure 3f).

Seasonal change in nutrient absorption rate of M. trifoliata

Seasonal changes in Rb absorption rate by excised *M. trifoliata* roots showed significant variation ($p < 0.001$; Figure 4), although fluctuation was great. The Rb absorption rate showed a maximum on 24 January (3.9 mg g^{-1} h^{-1}), and then fluctuated between 3.0-3.7 mg g^{-1} h^{-1} from January to May. Rb absorption rate showed a minimum value on 11 June (2.6 mg g^{-1} h^{-1}) and 23 July (2.5 mg g^{-1} h^{-1}), and then increased to 4.0 mg g^{-1} h^{-1} on 14 November.

Discussion

The leaf population of *Menyanthes trifoliata* in Mizorogaike Pond showed two peaks, in late May and early September (Figure 1), and this phenological property of *M. trifoliata* is not an observation unique to 1989 (Haraguchi 1993). One of the possible explanations why leaves of *M. trifoliata* in Mizorogaike Pond disappeared during the middle of the growing season is that disappearance of leaves has advantageous for dry matter production. According to Kikuzawa (1991), dry matter in leaves can be used for the formation of new organs such as reproductive parts or new leaves. Reproduction of the new high-quality leaves results in higher dry matter production than does the strategy to elongate the life of the old low-quality leaves, and hence the leaves of some plant species temporarily disappear in the summer season when the displacement of the leaves occurs. According to the carbon gain model developed by Kikuzawa (1991), leaf lifespan can be explained from the viewpoint of maximizing carbon gain during the period favorable for photosynthesis. The temporary foliage disappearance of *M. trifoliata* could be an adaptive strategy for the species for maximizing carbon gain in the warm-temperate climate, although a temporary leafless period during the growing season would not favorable for photosynthesis by *M. trifoliata* in Mizorogaike Pond.

Another possible explanation why the *M. trifoliata* in Mizorogaike Pond loses leaves within the middle of the growing season is the physiological effect of the rhizosphere environment on the plant. Although *M. trifoliata* is widely distributed and creates crowded and almost pure stands of relatively reduced (oxygen deficit) sites in Mizorogaike Pond (Haraguchi 1991b), the temporary decrease in Eh in the high-temperature season could cause the temporary defoliation of *M. trifoliata*.

The Eh of the surface peat showed a minimum value in June and this seasonal pattern of Eh change was commonly observed from 1985 to 1992 (Haraguchi 1991 a, b; Haraguchi 1995). A decrease in leaf population of *M. trifoliata* began in late May and this corresponds to a period just after the beginning of the Eh decrease of peat (Figure 1, Figure 2c). Eh began to increase in late July and the re-expansion of leaves begins in early August just after the beginning of the Eh increase (Figure 1, Figure 2c). According to the corresponding seasonal changes in foliage and Eh, leaf population in summer would be regulated by the redox properties of peat. A low Eh condition should inhibit *M. trifoliata* growth and cause leaf senescence.

The effects of Eh change on the foliage of *M. trifoliata* can be discussed based on the two processes: decreasing Eh and foliage in May-July, and increasing in August.

The Eh is determined by oxic-anoxic environments in peat soil, and Eh also determines the chemical environment in the peat. Under low dissolved oxygen conditions, Eh rapidly decreases because of the rapid consumption of dissolved oxygen (Wetzel 1983). Low Eh conditions usually inhibit the growth of plants (Crawford 1983; Rorison and Robinson 1986).

An increase in Eh corresponded to the leaf population increment. In the season of Eh increment, some species such as *Eriocaulon sikokianum* Maxim., *Isachne globosa* O. Kuntze and *Rhynchospora fauriei* Franch. showed rapid vegetative growth (Haraguchi, unpublished data). The foliage reappearance of *M. trifoliata*, however, was observed only in pure or almost pure habitats of *M. trifoliata*. In mixed stands with species which develop foliage above *M. trifoliata*, reappearance of foliage is not observed (Haraguchi 1993). In mixed stands with species such as *Phragmites australis* (cav.) Trin. ex. Steud., competition for space occupation between species would occur. Hence, *M. trifoliata* fails to develop its foliage in some mixed stands. Although the light environment could be one of the determinant factors of leaf expansion in August, high peat redox potential would be a requisite factor for the expansion of leaves in this season. Increase in root nutrient elements from August to September implies that nutrient absorption in the high Eh environment would be necessary for leaf expansion after leaf decay in summer season (Figure 3).

The low Eh of surface peat would cause the temporary senescence of the foliage of *M. trifoliata* in the peat mat. Minimum nutrient concentrations (K, Mg, Ca, Fe) of *M. trifoliata* root from June to August and the subsequent increase after August (Figure 3) imply that the anoxic condition or nutrient deficit of the plant root caused by the anoxic soil environment would cause the temporary senescence of the foliage of *M. trifoliata*. After August, the Eh increases and nutrient concentration in plant roots higher (Figure 2c, Figure 3). Improvement of nutritional conditions would lead to recovery of the foliage of *M. trifoliata*. The nutrient absorption rate of *M. trifoliata* evaluated by the Rb absorption rate of excised roots of *M. trifoliata* also showed minimum values in June and July (Figure 4). This implies that the peat redox properties affect the nutrient absorption activity by roots and the consequent foliage phenology of *M. trifoliata* at the study site.

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