Spatial variation of denitrification potential of grassland, windbreak forest, and riparian forest soils in an agricultural catchment in eastern Hokkaido, Japan

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Abstract
Riparian forest soils have a high denitrification potential (DP), but the effect of their spatial distribution on DP within a catchment has rarely been assessed. To provide insights into this effect, we evaluated the spatial distribution of DP and its control factors in the Shibetsu River Catchment (SRC), eastern Hokkaido, Japan. Soil samples from August 2008 were used in the lab to determine the DP (using the acetylene inhibition method) of grassland, windbreak forest, and riparian forest soils in the SRC. Soil DP ranged from 0.001 to 64 mg N kg soil^{-1} d^{-1} and followed a log-normal distribution. Soil DP was highest in the surface peat soil of a low-elevation riparian forest. The land-use type and soil depth both significantly affected soil DP, with higher DP in riparian forests and surface soils. Soil DP increased with increasing soil moisture content and water-extractable soil organic carbon (WESOC). The clay, moisture, and WESOC contents in the surface soils decreased significantly with increasing elevation, resulting in increased soil DP in low-elevation riparian forests. The stream NO_3^- concentration was significantly correlated with the upland (grassland and cropland) proportion (%) of the catchment's area, and the ratio of stream NO_3^- concentration to this proportion tended to be lower in low-elevation areas, indicating higher NO_3^- denitrification. We conclude that low-elevation riparian forest soils have a higher DP due to the higher moisture, clay, and WESOC contents of their soils, and that these factors control riverine NO_3^- export in the SRC.

Keywords: Denitrification, Riparian forest, Hotspot, Landscape, Watershed, Nitrogen cycle
Abbreviations: asl, above sea level; DP, denitrification potential; NNI, net nitrogen input; SRC, Shibetsu River Catchment; WESOC, water-extractable soil organic carbon.

1. Introduction
Human activities have greatly altered the nitrogen (N) cycle by accelerating the rate of anthropogenic N fixation in landscapes (Vitousek et al., 1997; Galloway and Cowling, 2002). Greater inputs of reactive N have increased crop production but have also lead to eutrophication of surface water, causing degradation of aquatic ecosystems and problems such as toxic algal blooms, loss of oxygen, depletion of fish populations, and biodiversity loss (Carpenter et al., 1998). Understanding the sources of N loading in water bodies is therefore essential to develop landscape-scale nutrient-management strategies (Boyer et al., 2002). However, non–point source pollution is difficult to

quantify because it generally encompasses large areas within drainage basins and involves more complex biotic and abiotic interactions than is the case for point-source pollution (Carpenter et al., 1998).

In general, riverine N export from watersheds does not exceed net N input (NNI) into terrestrial systems (Howarth et al., 1996; David and Gentry, 2000; Boyer et al., 2002, 2006; Hayakawa et al., 2009). These findings clearly indicate that whether N in terrestrial systems is surplus or missing depends on the balance between losses into the atmosphere by denitrification and changes in the N stock of soils and woody biomass (Howarth et al., 1996; Van Breemen et al., 2002). Denitrification is the conversion of NO_3^- into gaseous forms of N (nitrous oxide, N_2O; nitric oxide, NO; and dinitrogen gas, N_2), and is performed by particular groups of ubiquitous heterotrophic bacteria that have the ability to use NO_3^- as an electron acceptor during anaerobic respiration. The main factors controlling denitrification rates, therefore, are the supply of carbon (C), NO_3^-, and oxygen (Tiedje, 1994). Terrestrial-aquatic interfaces such as riparian forests have been interested in the role of buffers that reduce NO_3^- by denitrification which has been identified as the primary mechanism of NO_3^- removal because of carbon supply from vegetation (Hill and Cardaci, 2004), NO_3^- supply from adjacent agricultural land, and formation of anaerobic zone in low-elevation site (Harms and Grimm, 2008) in the forest, if the hydrology of the forest is appropriate (Burt et al., 1999; Vidon and Hill, 2004). Several studies have shown that groundwater NO_3^- concentrations decrease in riparian forests adjacent to uplands where the groundwater has been polluted by intensive agricultural management (Peterjohn and Correll, 1984; Lowrance, 1992; Hill, 1996). Balestrini et al. (2011) reported denitrification plays a dominant role in the NO_3^- depletion observed within the first few meters of a strip of vegetation. Soil texture (Groffman and Tiedje, 1989; Ullah and Faulkner, 2006), vegetation type (Hill and Cardaci, 2004; Dodla et al., 2008), microtopography (Harms and Grimm, 2008), hydrological pathways (Burt et al., 1999; Vidon and Hill, 2004), and their interactions can influence denitrification rates in the soil of a riparian forest.

This potential N removal function of riparian forest may have significance in N budget in a catchment scale. Thus, over the next decade, fundamental research on riparian zones is expected to allow extrapolations of denitrification in these areas to the overall catchment area (Burt et al., 2010). Next step in better understanding nitrogen cycling, therefore, will be to elucidate the spatial distribution and the relative importance of riparian denitrification, and its effect on stream N export at a catchment or regional scale. We assume that landscape position such as catchment-scale elevation, which can determine river flow, sedimentary architecture, and soil texture and moisture, may be a control factor of soil DP of riparian forest which interfaces river and land in a catchment. However, despite much effort and many studies which have shown the effect of elevation in small-scale on soil DP within riparian forests (Ullah and Faulkner, 2006; Harms and Grimm, 2008), limited research has been done on the effects of landscape position on ecosystem-wide spatial distribution of soil DP in a catchment scale.

Japan has steep topography, and strong variation in elevation can occur within a narrow area. This is likely to strongly affect the spatial variation of soil texture and the associated DP. The Konsen region of eastern Hokkaido is of particular interest because it is the largest dairy farming region in Japan, and there are inputs of N-containing materials, both from inorganic fertilizer and from animal manure. Milk production in this region accounted for 15.7% of the country's total production in 2003 (Statistics Department, 2004; Hokkaido Statistics and Information Office, 2004). Previous studies conducted in the Shibetsu River Catchment (SRC) of this region have shown that the stream NO_3^- concentration was correlated with the upland (grassland and cropland) proportion (%) in the drainage basins (Woli et al., 2004; Hayakawa et al., 2006). On the other hand, riverine N export accounted for only 27% of the NNI into the SRC, indicating that large amounts of N were either lost to denitrification or retained within the SRC (Hayakawa et al., 2009). Soil DP is higher in riparian forest than in grassland, suggesting that this forest was an important land use that affected the N cycle of the SRC (Hayakawa et al., 2006). However, it is not yet clear how the distribution of riparian forest within the watershed affects soil DP.

In the present study, we selected research sites within the three main land-use types in the SRC (riparian forest, grassland, and windbreak forest). Our objectives were to evaluate the spatial distribution of soil DP that resulted from these different land uses and to identify the primary control factors in the SRC.

2. Materials and Methods

2.1. Study area

The SRC study area is located in eastern Hokkaido, Japan (Fig. 1). The catchment covers 679 km^2. The region has a hemi-boreal climate, which is characterized by warm summers and cold winters. Precipitation averages 1147 mm yr^{-1}, of which roughly 70% occurs during the growing season, and the annual mean temperature is 5 °C, with the lowest mean monthly temperature in February (-8.3 °C) and the highest in August (18.0 °C), based on averages from 1978 to 2002 (Japan Meteorological Agency, 2007). The average maximum snow depth during the winter is 73 cm. The elevation in the SRC ranges from 4 to 1059 m above sea level (asl). The elevation in 50% of the area is less than 160 m, and only 8.5% of the area is higher than 500 m (the upper mountain areas). The main soil types (Soil Survey Staff, 2006) are Andisols (Thapic Melanudands) and Inceptisols (Andic Dystrudepts and Typic Dystrudepts), but Histosols (Typic Haplohemists) also occur in lowland areas near the Shibetsu River.

Most of the wetlands in the lower Shibetsu River have disappeared, leaving only 2 km^2 of wetland, as a result of river improvement work that was accompanied by a change in land use from wetland to grassland (Tachibana et al., 1997; Nakamura, 2003). The town of Nakashibetsu, which covers most of the SRC, has a large area of grassland, and dairy farming is the main occupation. Grassland (dominated by *Phleum pratense*) occupies more than 95% of the area of agricultural land, and the remainder is cultivated with maize, sugar beet, potato, and Japanese radish. A large area of 180-m-wide windbreak forest dominated by Japanese larch (*Larix kaempferi*) surrounds the grassland areas (Fig. 1). In the present study, "grassland" is defined as agricultural pasture which is managed for meadow and grazing. There are also riparian forests along the Shibetsu River, which occupy 9% of the STCs total area, and the vegetation of these forests is dominated by broadleaf trees such as Hokkaido willow (*Salix rorida*), alder (*Alnus japonica*), and magnolia (*Magnolia obovata*).

2.2. Soil sampling

We established sampling sites for the determination of soil DP at nine sites in each of the three main land-use types in the SRC (Fig. 1). The elevations at these sites ranged from 10 to 185 m asl. We defined the sites as high-, mid-, or low-elevation according to their elevation; high-elevation sample sites were at 117, 122, 129, 185 m asl; mid-elevation sample sites were at 55, 67, and 89 m asl; and low-elevation sample sites were at 10 and 18 m asl. The soil type at 10 m asl was a Histosol (Typic Haplohemist). Andisols (Thaptic Melanudands) dominated the sites at 18, 55, 67, and 89 m asl. Inceptisols were the main soil type at the 122 and 129 m asl sites (Andic Dystrudepts) and at the 117 and 185 m asl sites (Typic Dystrudepts).

Soil samples were obtained once in August 2008. We assumed that the one-time snapshot of DP values reflected a spatial variability of DP and their control factors, although their temporal variation was another important aspect of the discrete pattern of DP. Soil samples to a depth of 0.2 m (excluding the surface litter layer) were collected at five random locations in each land-use type at the nine sites using a 3.0-cm-diameter auger. Three samples were obtained at each site at depths of 0.2 to 0.5 m and 0.5 to 1.0 m. The reason for making this composite sample was to obtain an unbiased estimate of the mean of DP at each depth of a site, reducing the total cost of running analysis in the laboratory (Wollum, 1994). We could not obtain samples at depths from 0.5 to 1.0 m at the riparian forest site at an elevation of 129 m asl because of the soil's high stone content. To determine soil moisture content and bulk density, we obtained three intact soil cores from 7.5 to 12.5 cm depth (5.0 cm in diameter) and one from 0.2 to 0.5 m and 0.5 to 1.0 m depth (both 3.0 cm in diameter).

2.3. Measurement of soil DP

Soil DP was determined using the acetylene inhibition technique, which inhibits the final step in the conversion of N_2O gas into N_2 gas (Tiedje, 1994). To determine the difference among sites and depths in the amount of organic C available to the denitrifying organisms, we defined DP as the denitrification rate that occurred under anaerobic conditions with abundant NO_3-N at 25 °C. Samples of fresh, homogenized soil (15 g) were placed into 100-mL glass bottles. A 15-mL aliquot of solution containing nitrate (200 mg-N L^{-1} as KNO_3) and chloramphenicol (1 g L^{-1}) was added to the bottles. The bottles were evacuated and flushed four times with O_2-free N_2 to ensure anaerobic conditions, and acetylene (C_2H_2) gas was added to a final concentration of 10% v/v (10 kPa) in the headspace. We extracted the headspace gas with a gas-tight syringe at 2 and 4 h after adding the acetylene, and calculated denitrification rates from the linear portion of the curve for N_2O production as a function of time.
Soil DP was calculated by the following equations:

$$DP = \Delta N_g / (M \times \Delta t) \qquad \text{eq. (1)}$$

$$N_g = [\rho \times C \times (V_g + \alpha V_L)] \times [273 / (273 + T)] \qquad \text{eq. (2)}$$

where DP is the soil DP (mg N kg soil^{-1} day^{-1}), ΔN_g is the change in the weight of N_2O (N_g) in the incubation bottle (mg N_2O-N), M is the fresh weight of soil (kg), Δt is the incubation time (days), ρ is the density of N_2O under standard temperature and pressure (1.25 g N_2O-N L^{-1}), C is the concentration of N_2O (ppmv), V_g is the headspace of the incubation bottle (L), V_L is the liquid volume (L), α is the Bunsen coefficient (0.544 at 25 °C; Tiedje, 1994), and T is the temperature (°C) at which the gas samples were collected. Nitrous oxide was determined using a GC-14B gas chromatograph with an electron-capture detector (Shimadzu, Kyoto, Japan). Values of DP were reported on a soil dry-weight basis (mg N kg soil^{-1} day^{-1}). The DP per unit area in a 1-m-deep soil layer (kg N ha^{-1} m^{-1} d^{-1}) was calculated by summing the area-based DPs for the three depths, which were calculated using the bulk densities and the DPs per unit dry weight of soil.

2.4. Soil analysis

Soil organic C (total C) and N (total N) were determined using an NC-1000 analyzer (Sumigraph, Niihama, Japan). The fresh soil samples were extracted in distilled water (soil:water, 1:5 w/v), and the concentrations of water-extractable soil organic carbon (WESOC) and NO_3^- were determined using a total organic carbon analyzer (TOC, Shimadzu) and ion chromatography (QIC Analyzer; Dionex, Sunnyvale, CA, USA), respectively. Soil texture in the surface layer (to a depth of 0.2 m) was determined by means of particle size analysis. The particles that were larger in diameter than 20 μm (sand) were separated using a sieve, whereas those smaller than 20 μm were fractionated into 2- to 20-μm (silt) and < 2-μm (clay) fractions using the pipette method (Soil Science Society of America, 2002).

2.5. River water sampling and chemical analysis of water samples

River water sampling was conducted 6 to 14 times at 21 sites and the specific discharge (m^3 s^{-1} km^{-2}) of each site was also measured in the SRC from 2003 to 2008, following the procedure in our previous study (Hayakawa et al., 2006). After sampling, the water samples were stored on ice until they could be transported to the laboratory, and were then stored at 4 °C until analysis. The water sample was filtered through 0.2-µm membrane filters and the NO_3^- concentration was then determined as described in section 2.4. Using the upland proportion (%) at each site (Woli et al., 2004), the ratio of riverine NO_3^- concentration (mg N L^{-1}) to the upland proportion (%) was calculated.

2.6. Statistical analysis

Statistical analyses were performed using Excel Toukei 2010 for Windows (Social Survey Research Information Co. Ltd., Tokyo, Japan). We used two-way ANOVA followed by Tukey's multiple-comparison test to identify significant differences among land-use types and soil depths in DP and soil physical and chemical properties. Three-way ANOVA was also used to identify significant differences among land-use types, soil depths, and elevations.

3. Results

3.1. Soil properties

Table 1 summarizes the physical and chemical properties of the soils for each land use and soil depth. ANOVA showed that both the land-use type and the soil depth significantly affected some soil properties, but that there was no interaction between land-use type and soil depth for any of the soil properties. Soil bulk density was significantly higher in the grassland (0.88 ± 0.22 g cm^{-3}; all values are mean ± SD unless otherwise noted) and increased significantly with increasing depth. Soil moisture did not differ significantly among the land-use types or soil depths. Total N and C contents did not differ significantly among land-use types, but were significantly higher at the soil surface than at greater depths. The soil C/N ratio was significantly lower in riparian forest than in windbreak forest, but did not differ significantly between the grassland and the two forest types or between the soil depths. Soil NO_3^- content did not differ significantly among the three land-use types, but was significantly higher in the surface soil layer (19 ± 33 mg kg $soil^{-1}$). Soil NH_4^+ content did not differ significantly among the three land-use types and the soil depths, although it tended to be higher in windbreak forest. The WESOC content was significantly lower in the grassland and in the soil layer from 0.5 m to 1.0 m in depth. The WESOC content was significantly correlated with soil bulk density (r = -0.489, $P < 0.01$, n = 80).

3.2. Effects of land-use type and soil depth on soil DP

Soil DPs ranged from 0.001 to 64 mg N kg $soil^{-1}$ d^{-1} (Fig. 2) and followed a log-normal distribution ($P > 0.05$, Shapiro-Wilk test: Fig. 3). The highest DP value was observed in a surface peat soil to a depth of 0.2 m in a downstream riparian forest (at 56 m asl). The ANOVA results showed that both the land-use type and the soil depth significantly affected soil DP ($P < 0.01$) and that there was no significant interaction between land-use type and soil depth (P = 0.527). Tukey's multiple-comparison test showed that DP was significantly higher in the riparian forest soil and in the surface soil to a depth of 0.2 m (Table 2).

3.3. Effects of soil properties on soil DP

Table 3 shows the values of Pearson's correlation coefficient for the relationships among all depths of the main soil properties and log(DP). For all land-use types combined, soil DP was significantly correlated with soil bulk density ($P < 0.01$, r = -0.36) and with the soil gravimetric water (r = 0.24, $P < 0.05$), total N (r = 0.34, P <

0.01), total C ($r = 0.26$, $P < 0.05$), NO_3^- ($r = 0.37$, $P < 0.01$), and WESOC ($r = 0.39$, $P < 0.01$) contents. Especially in the riparian forest, soil DP increased significantly with increasing soil gravimetric water ($r = 0.56$, $P < 0.01$) and WESOC ($r = 0.57$, $P < 0.01$) contents. On the other hand, soil DP in the windbreak forest was significantly correlated with the soil bulk density ($r = -0.46$, $P < 0.05$), and with the soil total N ($r = 0.46$, $P < 0.05$) and NO_3-N ($r = 0.48$, $P < 0.05$) contents. In the grassland, no soil parameters were significantly correlated with soil DP. In general, higher DP occurred at higher soil gravimetric water and WESOC contents in the riparian forests (Fig. 4). The WESOC content was significantly correlated with the gravimetric water content ($r = 0.572$, $P < 0.01$, $n = 80$).

3.4. Effects of land-use position on soil properties and soil DP

The ANOVA results showed that land-use type, soil depth, and elevation all significantly ($P < 0.01$) affected soil DP, and there was a significant ($P < 0.05$) interaction between land-use type and elevation (Table 4). Table 5 shows the Pearson's correlation coefficients for the relationships between various parameters and elevation. The elevation of the sample site was significantly correlated with several soil physical and chemical properties. Elevation was negatively correlated with the clay ($r = -0.47$, $P < 0.05$) and silt ($r = -0.56$, $P < 0.01$) contents and significantly positively correlated with the sand content ($r = 0.58$, $P < 0.01$) in the surface soil to a depth of 0.2 m. Elevation was also significantly negatively correlated with the soil gravimetric water ($r = -0.46$, $P < 0.05$) and WESOC ($r = -0.37$, $P < 0.10$) contents. The three-dimensional plots of these relationships clearly showed that elevation affected the clay and WESOC contents of the surface soils (Fig. 5). On the other hand, we found no significant correlations between elevation and soil DP except for the windbreak forests (Table 5). However, DP of the surface soils in the riparian forests tended to be negatively correlated with elevation, in contrast to the generally positive correlations for the soils of the grasslands and the windbreak forests (Table 5). The correlations became marginally significant for the riparian forest ($r = -0.65$, $P < 0.10$, $n = 8$) when the lowest DP value (for the site at an elevation of 67 m asl) was excluded. The DP per unit area was also marginally significantly correlated with elevation when this outlier was excluded ($r = -0.69$, $P < 0.10$, $n = 7$). The three-dimensional plot showed that soil DP tended to decrease with increasing elevation and increase with increasing soil water content in the riparian forest (Fig. 6).

Figure 7 shows the spatial distribution of soil DP and the ratio of riverine NO_3^- concentration (mg N L^{-1}) to the upland proportion (%) in the SRC. The mean riverine NO_3^- concentration increased significantly with increasing upland proportion ($r = 0.915$, $P < 0.01$, $n = 25$). Although the riverine NO_3^- / upland proportion ratio tended to decrease at the downstream sites, soil DP in the riparian forest tended to increase at the low-elevation sites.

4. Discussion

4.1. Effects of soil properties and land-use types on soil DP

The soil moisture and WESOC contents were significantly positively correlated with DP (Table 3, Fig. 4), possibly because high moisture and WESOC tend to produce anaerobic conditions (Burgin et al., 2010) and a large carbon source, respectively, for the denitrifying bacteria. These findings confirm the results of previous research (Hayakawa et al., 2006; Ullah and Faulkner, 2006). Significantly greater soil DP values were found in the riparian forests than in the windbreak forests and the grasslands (Table 2), and the correlations with soil moisture and WESOC were stronger for the riparian forests (Table 3). One reason for the difference was the high quantity of WESOC in the riparian forests (Tables 1, 3), although the quality of the organic carbon can also affect the DP (Hill and Cardaci, 2004; Dodla et al., 2008). The WESOC content was a better predictor of soil DP than the total C content (Table 3), which indicated that the water-soluble carbon was important for the denitrifying bacteria, especially in the riparian forests.

Litter from different plant species supplies different amounts and qualities of organic carbon to the denitrifying bacteria, and can thus affect DP (Bastviken et al., 2007). Dodla et al. (2008) reported that labile organic C, which was estimated from the total gaseous C (CO_2-C + CH_4-C) released during a 5-day incubation, was significantly correlated with DP at 2 mg NO_3^--N L^{-1}, whereas the content of phenolic compounds was negatively correlated with DP. The presence of phenolic compounds indicated the presence of lignin in the plant materials. The litter decomposition rate is faster with a low ratio of lignin to N in the litter (Taylor et al., 1991; Prescott, 2010). Since the windbreak forests in the SRC are dominated by Japanese larch, which generally has a high lignin content, whereas the riparian forests are dominated by broadleaf trees with a low lignin content, the quality of the organic carbon supplied to the soil would differ between the two forests. Therefore, the quality of WESOC would also differ and this would affect DP, although we found no significant difference in the WESOC contents of the two forests (Table 1).

The soil NO_3^- content, which might also have affected the activity of the denitrifying bacteria, can also explain the difference in DP among the land-use types (Tables 1, 3). However, the variation in the soil NO_3^- content in the riparian forest was high (Table 1; CV = 251%) as a result of the presence of an outlier. If the outlier was excluded, the mean value and the variation in the riparian forest (6.9 ± 8.0 mg N kg soil^{-1}) was similar to those in the windbreak forest (6.3 ± 8.0 mg N kg soil^{-1}).

In the windbreak forests, soil N significantly affected DP (Table 3), indicating that N was a limiting factor for DP in this forest type. Nitrogen might be supplied to the forest by atmospheric deposition and would influence N cycling by enhancing the activity of denitrifying bacteria. In the SRC, a large amount of NH_3 is input in the form of cattle manure and inorganic fertilizer, with the total input estimated at 20 kg N ha^{-1} y^{-1} (Hayakawa et al., 2009). This nitrogen source may be trapped at the edges of the grassland, since windbreak forests can function as traps and concentrators for wind-borne nutrients and pollutants (Weathers et al., 2001), and the increased nitrogen input might increase microbial nitrification and denitrification. Spatial variation in the N input to the windbreak forests might therefore cause the variation in DP.

In the grasslands, mean soil DP was the lowest of the three land-use types, likely because of the significantly higher bulk density and significantly lower WESOC content of the grassland soil (Table 1). There was a significant negative correlation between bulk density and WESOC content in the grassland soil (r = 0.480, P < 0.05, n = 27). Higher bulk density may restrict the supply of nutrients from litter by inhibiting root growth, resulting in a low WESOC content and a low DP. Jin et al. (2010) reported that soil DP in grassland was significantly higher in root-mat soil (0 to 2.5 cm) than in mineral soil (2.5 to 5.0 cm). In the grassland soil, denitrification would therefore occur in a limited surface layer compared with the forest sites; because labile organic C regulates soil DP, the soil parameters in the grasslands would be weakly correlated or not correlated with soil DP in the present study (Table 3).

4.2. Effects of landscape position on soil DP

Our data clearly showed that soil texture and moisture content were affected by landscape position (Table 5, Fig. 5). In general, fine-textured deposits accumulate in low-elevation areas because they are transported in percolating water and in surface flows. Such deposits would help soils to retain water as a result of decreased permeability and the high surface area of these particles. This would create anaerobic conditions suitable for denitrification in the low-elevation soils and would thereby affect soil DP (Fig. 6). Previous studies have reported that soil texture significantly influences DP (Groffman and Tiedje, 1989; Ullah and Faulkner, 2006). Ullah and Faulkner (2006) reported that low-elevation clay soils in wetlands exhibited 6.3 times the DP of high-elevation silt loam soils in a small catchment (0.25 km^2) with a maximum elevation difference of 5.5 m. Harms and Grimm (2008) also showed that low-elevation sites were hotspots of denitrification at a microtopographic scale. The present study, in a larger catchment (>100 km^2) indicated that landscape position (elevation) affected the suitability of the water environment for denitrification through its effect on soil texture. Only in the riparian forest soils was DP negatively correlated with elevation, and the correlation was only marginally significant ($P < 0.10$) even when an outlier was excluded (Table 5), indicating that landscape position may control soil DP in the riparian forest. Landscape also affected the soil type of the riparian forest by controlling the soil texture and water environment. Histosols (10 m asl), which had the highest DP and were distributed in the downstream regions of the SRC, clearly reflected the landscape position in the watershed.

However, soil DP in the riparian forest was not always strongly correlated with elevation (Table 5), possibly because site-specific conditions such as microtopography (Harms and Grimm, 2008), soil texture (Groffman and Tiedje, 1989), litter quality and quantity (Dodla et al., 2008), hydrological pathways (Vidon and Hill, 2004), and their interactions were more important in the riparian forest, leading to the presence of biogeochemical hotspots with disproportionately high reaction rates relative to the surrounding matrix (McClain et al., 2003). The riparian forest where the highest DP was observed would be such a hotspot, possibly because it also had the highest WESOC content. In contrast, the lowest riparian forest site, at an elevation of 67 m asl, would be a cold spot for DP (0.45 kg N ha^{-1} d^{-1}), although the elevation was not particularly high in absolute terms and the WESOC content was not particularly low. On the other hand, elevation did not significantly affect soil DP in the grasslands and positively correlated with soil DP in the windbreak forests (Table 5), emphasizing the importance of location for the riparian forest. Riparian zones are sensitive locations because they occur at the interface between slopes and rivers, and therefore play important roles in flood attenuation and sedimentation control (Naiman and Décamps, 1997), both of which might affect DP compared to the grasslands and windbreak forests. Both location with respect to the other land-use types and landscape position within the catchment will soil DP in the riparian forests.

4.3. Effects of denitrification in the riparian forests on riverine nitrogen export

In the low-elevation riparian forest of the SRC, soil DP tended to be higher and the stream NO_3^- concentration / upland proportion tended to be lower (Fig. 7), suggesting greater removal of NO_3^- by this forest. Riverine N export accounted for only 27% of the NNI estimated in a previous study using a N budget model of the SRC, which indicated that a large amount of missing N (55 kg N ha^{-1} y^{-1}) was denitrified or retained in the SRC (Hayakawa et al., 2009). Riverine NO_3^- export per unit NNI measured in five drainage basins in the SRC ranged from 0.12 to 0.35, and was lower in the downstream basins (Hayakawa et al., 2009), which would also support high NO_3^- removal in the low-elevation areas. In the present study, the highest DP was observed in the low-elevation riparian forest, and amounted to 55 kg N ha^{-1} m^{-1} d^{-1}, and the mean DP in the riparian forest was 23 ± 18 kg N ha^{-1} m^{-1} d^{-1} (n = 8). Although the DP measured in the lab in the present study is only a one-time snapshot of potential value, one meter thickness soil might have sufficiently high potential activity of denitrification, which was several hundred times larger than the missing N reported previously. Actual denitridication depends mainly on WFPS, NO_3^- concentration and SOC decomposition and decreases to almost zero when one of those factors decreases enough (Parto et al. , 1996; Bell et al., 2012). Therefore, if sufficient condition for denitrification was provided even in the small area of watershed like riparian forests, the missing N could be denitrified at the area. Jiang et al. (2010) used a hydrological process analysis in the SRC to show that NO_3^-, which originated in the near-surface soil layer associated with a rising groundwater table in downstream areas, was mainly flushed from the soil by subsurface runoff during storm events. This finding also supports the belief that NO_3^- supplied from shallow groundwater to surface or subsurface soils after storm events can be denitrified during "hot moments" in the riparian forest, and especially at the low-elevation riparian forest. Combining these findings with a mass-balance approach, synoptic water sampling, hydrological analysis, and the measurement of soil DPs would clarify whether denitrification in the riparian forest (especially in the low-elevation forest) controls riverine N export and would explain part of the missing N in the SRC.

However, other factors may also be responsible for the decreased NO_3^- at low-elevation sites. These include dilution effects, the proportion of the riparian forest area in each drainage basin, and the possibility of in-stream denitrification. Dilution effects in low-elevation areas do not appear to be highly significant because the correlation between the stream NO_3^- concentration / upland proportion and the specific discharge was negative but not significant (r = -0.346, P = 0.124, n = 21). The proportion of riparian forest area in each drainage basin did not seem to vary among the drainages in the SRC, so it cannot explain the difference in NO_3^- concentrations among the drainages. In-stream denitrification may also contribute to decline NO_3^- concentration in downstream regions (Inwood et al., 2005; Garnier et al., 2010), and should therefore be investigated in the SRC.

Additional studies are needed to determine the role of organic matter quality in the riparian forest and its spatial distribution in the SRC to evaluate the importance of heterotrophic denitrification in the fate of N surpluses and to predict denitrification at a catchment scale (Oehler et al., 2009). Combining the spatial distribution of labile C with geochemical data such as solid-associated Fe(II) and pyrite, which can be electron donors for denitrification (Craig et al., 2010), would also help us to evaluate ecosystem-wide denitrification. Monitoring denitrification in the SRC in the field over a longer period to detect hot moments would also provide important insights into denitrification, since soil moisture, soil NO_3^- content, and the supply of organic matter to soil, all of which control DP, will vary significantly both within and between seasons.

5. Conclusions

Our results showed that the riparian forest soils had high DP, which was significantly controlled by the soil moisture and WESOC contents. Landscape position (elevation) affected the suitability of the water environment for denitrification by controlling the soil texture. The clay, moisture, and WESOC contents of the surface soils decreased significantly with increasing elevation, resulting in increased soil DP in the low-elevation riparian forests. Although stream NO_3^- concentrations were significantly correlated with the upland proportion in the catchment, the stream NO_3^- concentration / upland proportion ratio tended to be lower at the low-elevation sample sites, indicating greater denitrification at the low-elevation sites. Combining the findings of a mass-balance approach, synoptic water sampling, hydrological analysis, and soil DP measurements would clarify how denitrification in the riparian forest, and especially in the low-elevation forests, controls riverine N export in the SRC.

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Figures

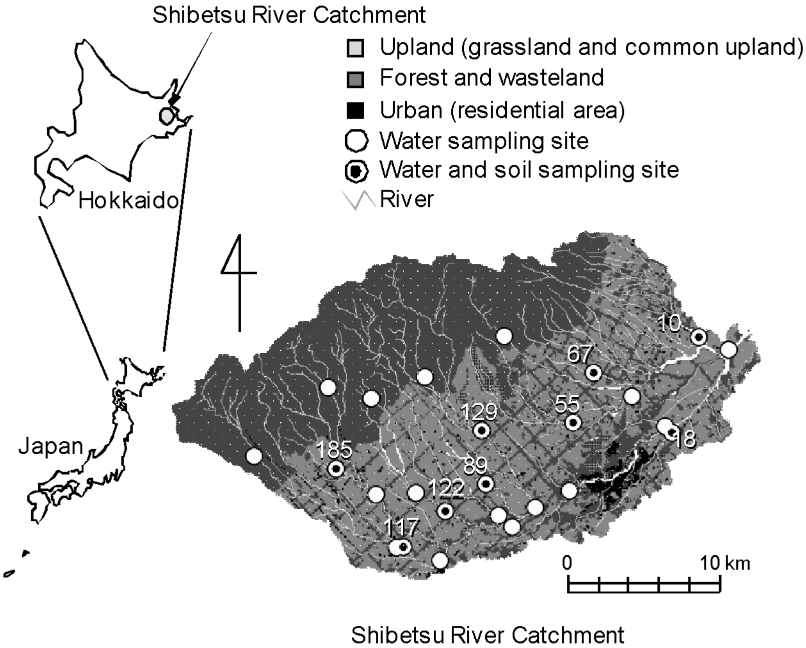

Fig. 1. Land-use types and the sampling sites in the Shibetsu River Catchment, Hokkaido, Japan. The white circles inside the catchment represent the water sampling points. The black circles within white circle represent the water and soil sampling points and their elevation (m). There is a large area of windbreak forest surrounding the grassland.

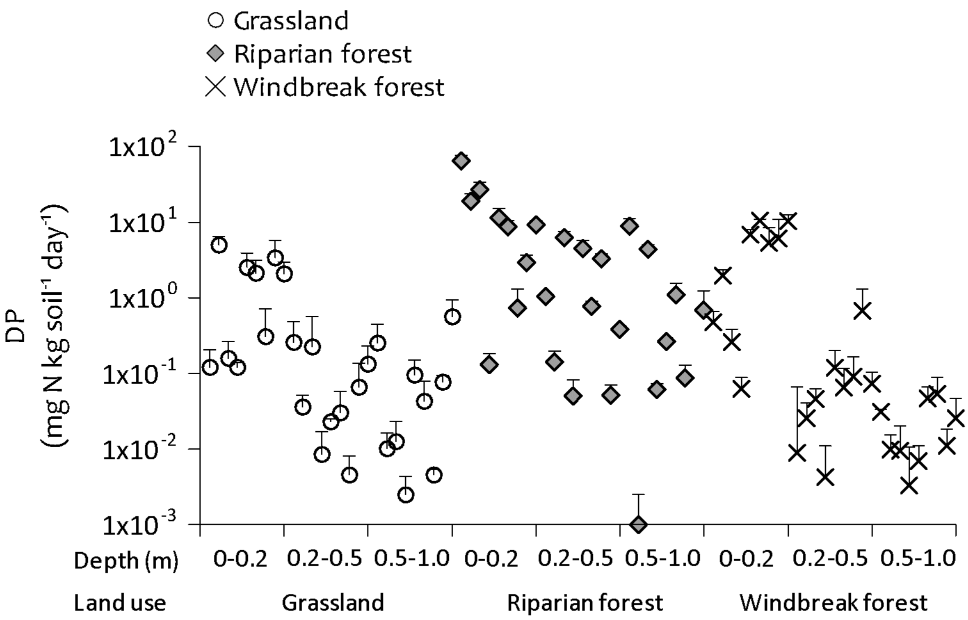

Fig. 2. Soil denitrification potential (DP) of the three land-use types and three soil depths. Values represent means and standard deviations (n = 3). The values for a given land-use type and depth are arranged in order of increasing elevation of the sampling site.

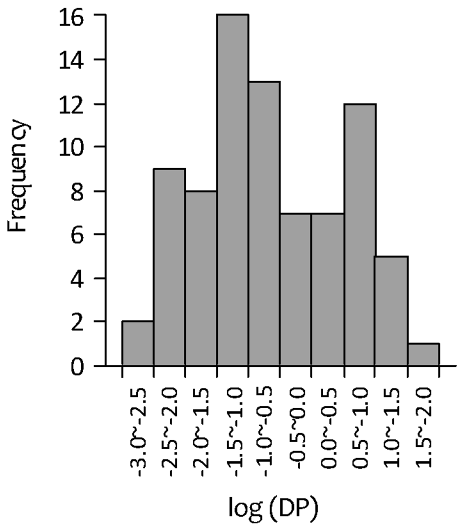

Fig. 3. Frequency distribution of the logarithm of the soil denitrification potential (DP; mg N kg soil^{-1} d^{-1}).

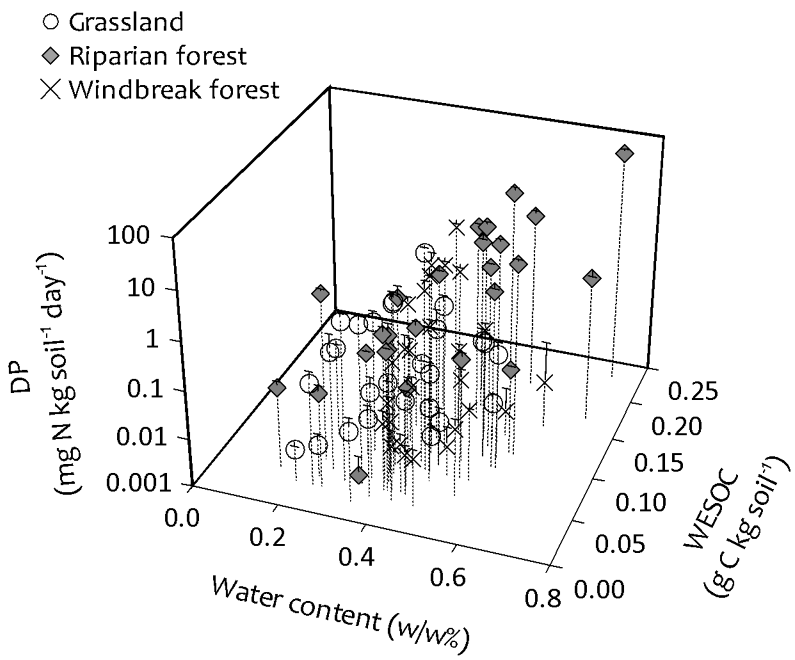

Fig. 4. Soil denitrification potential (DP) as a function of soil gravimetric water content and water-extractable soil organic carbon (WESOC) content. Values represent means and standard deviations (n = 3).

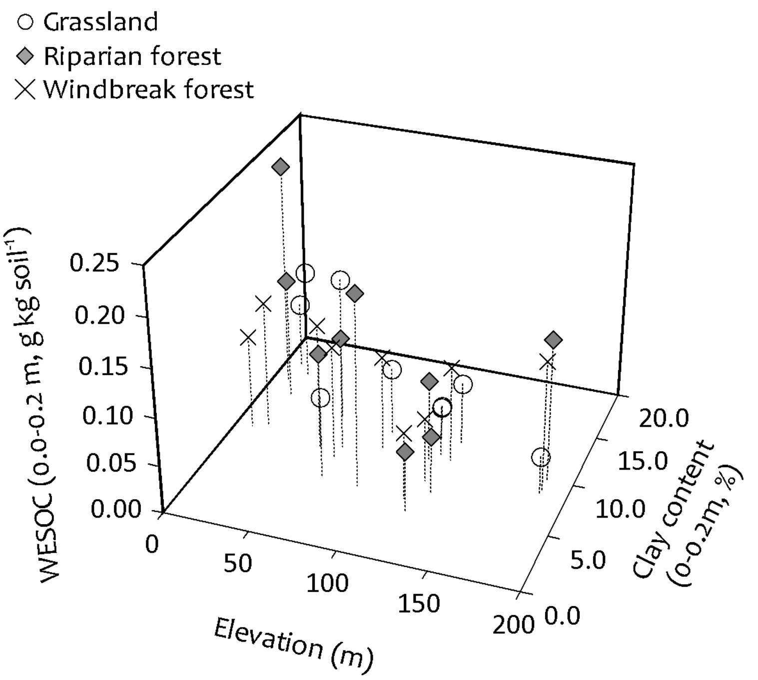

Fig. 5. Water-extractable soil organic carbon (WESOC) content to a depth of 0.2 m as a function of elevation and clay content to a depth of 0.2 m for the three land-use types.

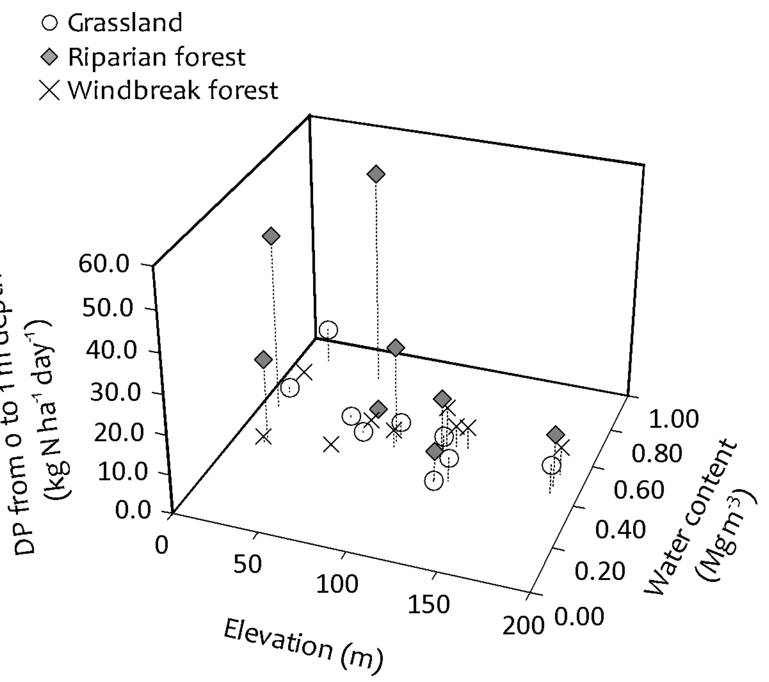

Fig. 6. Soil denitrification potential (DP) per unit area to a depth of 1.0 m as a function of elevation and water content for the three land-use types. One sample plot for the riparian forest was excluded because we could not obtain a sample at depths of 0.5 m to 1.0 m at the site with an elevation of 129 m asl.

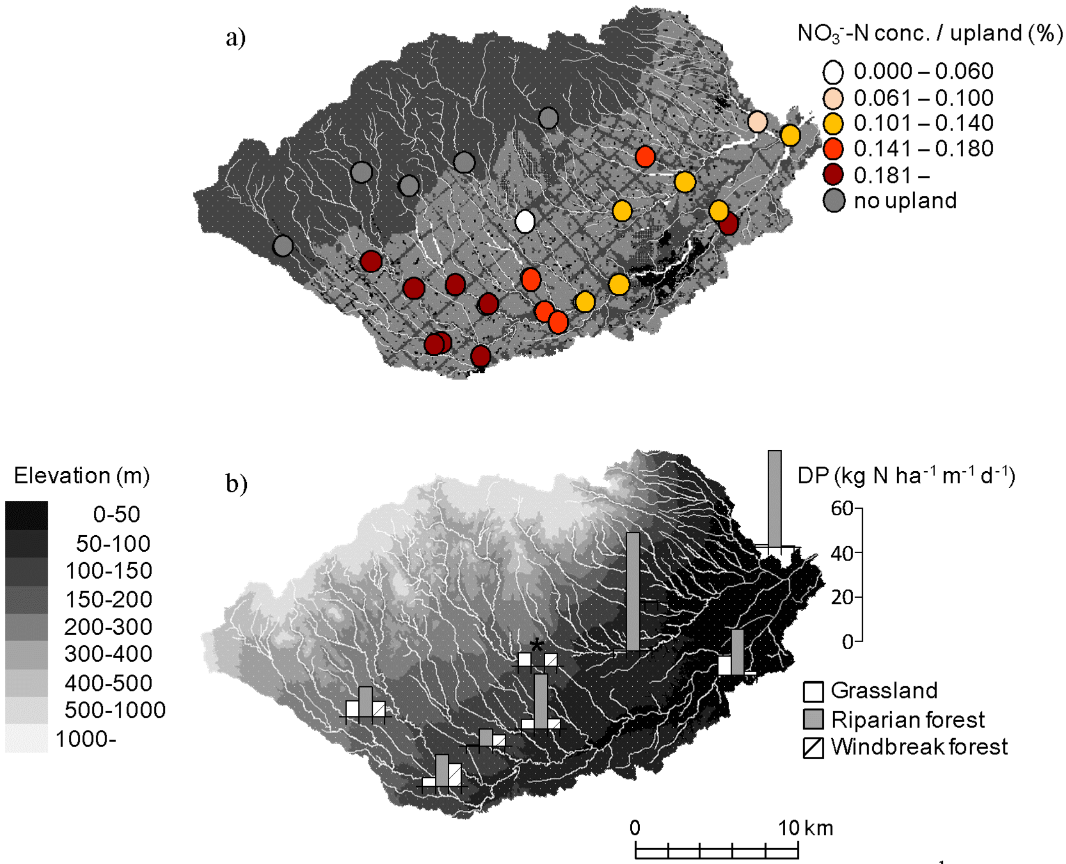

Fig. 7. Spatial distribution of (a) the riverine NO_3^- concentration (mg N L^{-1}) divided by the upland proportion (%) and (b) the soil denitrification potential (DP; kg N ha^{-1} m^{-1} d^{-1}) for the three land-use types in the Shibetsu River Catchment. * indicates a sample plot in the riparian forest for which no data was available.

Table 1 Mean physical and chemical properties of soils from the three land uses and three depths. The values in parentheses represent standard deviations.

Soil parameters	Land-use type: Grassland	Land-use type: Riparian forest	Land-use type: Windbreak forest	Depth (m): 0.0-0.2	Depth (m): 0.2-0.5	Depth (m): 0.5-1.0
Bulk density (g cm^{-3})	0.88 (0.22) a	0.65 (0.23) b	0.62 (0.24) b	0.60 (0.23) A	0.76 (0.27) B	0.79 (0.22) B
Gravimetric water content (g g^{-1})	0.38 (0.10)	0.45 (0.14)	0.45 (0.07)	0.43 (0.10)	0.43 (0.12)	0.43 (0.12)
Clay, 0.0-0.2 m (%)	12 (3.3)	9.2 (3.3)	8.7 (1.4)	- (-)	- (-)	- (-)
Silt, 0.0-0.2 m (%)	36 (6.4) ab	27 (12) a	39 (2.0) b	- (-)	- (-)	- (-)
Sand, 0.0-0.2 m (%)	52 (8.4)	64 (16)	52 (3.0)	- (-)	- (-)	- (-)
pH	5.5 (0.55)	5.4 (0.53)	5.2 (0.44)	5.2 (0.55)	5.4 (0.54)	5.5 (0.44)
Total N (kg Mg^{-1})	3.2 (2.0)	3.7 (2.8)	4.0 (1.9)	4.9 (2.4) A	3.5 (2.1) B	2.6 (1.6) B
Total C (kg Mg^{-1})	45 (32)	48 (37)	58 (28)	65 (31) A	49 (35) AB	36 (26) B
C/N	13 (2.4) ab	12 (3.0) a	14 (1.2) b	13 (1.2)	13 (2.3)	13 (3.5)
NO_3-N (mg kg $soil^{-1}$)	8.5 (10)	13 (33)	6.3 (8.0)	19 (33) A	5.1 (5.3) B	4.1 (3.7) B
NH_4-N (mg kg $soil^{-1}$)	2.4 (1.1)	3.9 (3.6)	20 (82)	3.1 (1.4)	20 (82)	3.3 (3.5)
WESOC (mg kg $soil^{-1}$)	74 (37) a	104 (55) b	83 (37) b	103 (46) A	85 (45) A	72 (38) B

Values of a parameter followed by different letters differ significantly among the land-use types (a, ab, b) and soil depths (A, AB, B) ($p < 0.05$; Tukey's multiple-comparison test). We found no significant interaction between land-use type and depth for all parameters.

Table 2 Mean logarithm of the soil denitrification potential (DP; mg N kg soil^{-1} d^{-1}) for the three land-use types and three soil depths. The values in parentheses represent standard deviations

Land-use type	Depth (m) 0-0.2		0.2-0.5		0.5-1.0		All	
Grassland	-0.10	(0.68)	-1.37	(0.61)	-1.46	(0.80)	-0.98 (0.92)	a
Riparian forest	0.77	(0.83)	-0.22	(0.80)	-0.55	(1.24)	0.02 (1.09)	b
Windbreak forest	0.28	(0.81)	-1.31	(0.64)	-1.82	(0.41)	-0.95 (1.10)	a
All	0.32 (0.83)	A	-0.97 (0.85)	B	-1.30 (0.99)	B		

Values of a parameter followed by different letters differ significantly among the land-use types (a, ab, b) and soil depths (A, AB, B) ($P < 0.05$; Tukey's multiple-comparison test). We found no significant interaction between the land-use type and soil depth ($P = 0.527$).

Table 3 Pearson's correlation coefficients among all depths of soil properties and the logarithm of the soil denitrification potential (DP).

Land-use type	*n*	Bulk density		Gravimetric water content		pH	Total N		Total C		C/N	NO_3-N		WESOC	
All land-use types	80	-0.36	**	0.24	*	-0.05	0.34	**	0.26	*	-0.18	0.37	**	0.39	**
Grassland	27	0.15		-0.34		0.01	0.03		-0.07		-0.17	0.23		-0.26	
Riparian forest	26	-0.33		0.56	**	-0.09	0.35		0.31		0.03	0.38		0.57	**
Windbreak forest	27	-0.46	*	-0.21		-0.07	0.46	*	0.38		-0.27	0.48	*	0.28	

**, $P < 0.01$; *, $P < 0.05$

Table 4 Results of the three-way ANOVA for the effects of land-use type, soil depth, and elevation and their interactions on the logarithm of the soil denitrification potential (DP; mg N kg soil^{-1} d^{-1}).

Factor	*df*	Mean Sq.	*F* value	*P*	
Land-use type	2	8.4	28.9	<0.001	**
Depth	2	19.2	65.9	<0.001	**
Elevation	8	1.85	6.33	<0.001	**
Land-use type × Depth	4	0.50	1.70	0.175	
Land-use type × Elevation	16	0.70	2.39	0.017	*
Depth × Elevation	16	0.48	1.66	0.109	
Land-use type × Depth × Elevation	0	-			
Residuals	32	0.29			

df, degrees of freedom; Mean Sq., mean square
**, $P < 0.01$; *, $P < 0.05$

Table 5 Pearson's correlation coefficients for the relationships between elevation and the soil parameters. DP, soil denitrification potential.

	n	Correlation coefficient	
Bulk density, 0.0-0.2 m (g cm^{-3})	27	0.24	
Water content (Mg m^{-3})	26	-0.47	*
Gravimetric water content, 0.0-0.2 m (g g^{-1})	27	-0.46	*
Clay, 0.0-0.2 m (%)	27	-0.47	*
Silt, 0.0-0.2 m (%)	27	-0.56	**
Sand, 0.0-0.2 m (%)	27	0.58	**
WESOC, 0.0-0.2m (mg kg soil^{-1})	27	-0.37	+
log(DP)			
All types, 0.0-0.2 m (mg N kg soil^{-1} d^{-1})	27	0.19	
All types, 0.0-1.0 m (kg N ha^{-1} d^{-1})	27	0.22	
Grassland, 0.0-0.2 m (mg N kg soil^{-1} d^{-1})	9	0.37	
Riparian forest, 0.0-0.2 m (mg N kg soil^{-1} d^{-1})	9	-0.32	
Windbreak forest, 0.0-0.2 m (mg N kg soil^{-1} d^{-1})	9	0.65	+
Grassland, 0.0-1.0 m (kg N ha^{-1} d^{-1})	9	0.30	
Riparian forest, 0.0-1.0 m (kg N ha^{-1} d^{-1})	8	-0.19	
Windbreak forest, 0.0-1.0 m (kg N ha^{-1} d^{-1})	9	0.66	+
Riparian forest, 0.0-0.2 m (mg N kg soil^{-1} d^{-1})	8‡	-0.65	+
Riparian forest, 0.0-1.0 m (kg N ha^{-1} d^{-1})	7‡	-0.79	+

‡, The lowest DP value, which occurred at the site at an elevation of 67 m asl, was excluded as an outlier.
**, $P < 0.01$; *, $P < 0.05$; +, $P < 0.1$