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Aluminum and acidity suppress microbial activity and biomass in acidic forest soils

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ABSTRACT

To evaluate the effects of aluminum (Al) toxicity on microbial processes in acidic forest soils, we determined microbial biomass, soil enzyme activities associated with carbon (C), nitrogen, and phosphorus cycling (β-D-glucosidase and polyphenol oxidase, L-asparaginase, and acid phosphatase, respectively), exchangeable base cations, soluble and exchangeable Al ($CaCl_2$–Al and KCl–Al), and organically bound (Al_p and Fe_p) and both organically bound and noncrystalline forms (Al_o and Fe_o) of Al and iron (Fe) in Japanese forest soils (25 Inceptisols, seven allophanic Andisols, and eight nonallophanic Andisols). The exchangeable calcium (Ca) and magnesium (Mg) concentrations decreased significantly with decreasing soil pH. In contrast, the KCl–Al and $CaCl_2$–Al concentrations exponentially increased with decreasing pH, and the increase was steeper in Andisols than in Inceptisols. The Al_p concentration also increased exponentially with decreasing soil pH in Andisols, and the Fe_p concentration increased in Andisols and Inceptisols, whereas the concentration of the noncrystalline Al pool (Al_o – Al_p) decreased with decreasing pH in Andisols. We showed that the β-D-glucosidase, polyphenol oxidase, and acid phosphatase activities were mainly suppressed by soluble and exchangeable Al, whereas L-asparaginase activity was depressed by soil pH. In Andisols, a negative effect of organically bound Al and Fe on the biomass C/organic C ratio was observed. These results suggest that Al toxicity and acidity repressed soil enzyme activities, leading to suppressed microbially mediated nutrient cycling, and that Al toxicity and a reduced availability of organic matter, as a result of Al and Fe binding, may protect a substantial pool of organic C from microbial degradation in acidic forest soils.

1. Introduction

Acidity develops naturally in soils of relatively humid areas, including Japan, and acidic soils with pH < 5.5 account for approximately 30% of the world's ice-free land area (Hodson and Donner, 2013). Acidification in forest soils is accelerated by acid deposition from anthropogenic sources. In North America and Europe, acid deposition damaged forest and aquatic ecosystems during the later decades of the 20th century (Miller, 2002). Growth-limiting factors for plants in acidic soils include aluminum (Al) and hydrogen (H) toxicities, as well as deficiencies of essential elements such as calcium (Ca) and magnesium (Mg) (Foy, 1984); Al toxicity is considered to be one of the most deleterious factors for plant growth in acidic soils. According to Foy (1984), Al toxicity is more important than toxicity related to pH in limiting the growth of higher plants in many acidic soils (pH > 4.0).

Because of efforts to reduce sulfur (S) and nitrogen (N) emissions starting in the mid-to-late 20th century, acid deposition has been largely reduced in North America and Europe (Lajtha and Jones, 2013), although there are still widespread areas of ongoing acidification in forest soils in the United States (Greaver et al., 2012). In stark contrast, rates of S deposition and N deposition have recently increased in Japan: the annual increase rate was 1.69% y^{-1}

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for non-seasalt SO_4^{2-} wet deposition during 1981–2005 (Kuribayashi et al., 2012) and ~4% y^{-1} for NO_3^- wet deposition during 1994–2008 (Morino et al., 2011). The average pH of precipitation from 2003 to 2007 was 4.68 in Japan, and strong acidic precipitation (pH < 4.0) accounted for 4.5% of the total precipitation at 14 sites where daily sampling has been conducted (Ministry of the Environment, 2009). Transboundary pollution has largely accounted for the increased acid deposition in Japan. China was estimated to account for 34% of the S deposition from 1981 to 1985 and 51% from 2001 to 2005 (Kuribayashi et al., 2012), while it accounted for 29–35% of the NO_3^- wet deposition from 1989 to 1993 and 43–61% from 2004 to 2008 (Morino et al., 2011) in Japan. Although no apparent impacts of acid deposition on ecosystems have been observed in Japan, in the future, negative impacts might occur if the acid deposition rate remains at its current level (Ministry of the Environment, 2006).

Al toxicity to plants, especially crops, has been studied extensively (Matsumoto, 2000). In contrast, although many studies have addressed the effects of acid deposition on soil microorganisms, most of the studies have only examined the effects of increased acidity (Wolters and Schaefer, 1994), and little information exists regarding the impact of elevated levels of soluble and exchangeable Al on soil microbial processes in forest soils (e.g., Illmer et al., 1995; Kanazawa and Kunito, 1996; Joner et al., 2005). In almost all forest ecosystems with acidic soils, woody plants do not show apparent stress symptoms in response to Al toxicity (Brunner and Sperisen, 2013), but soil microorganisms and microbially mediated nutrient cycling might be affected by Al toxicity. Kraal et al. (2009) reported that the addition of Al suppressed soil respiration, nitrification, and the microbial uptake of NH_4^+ in pine litter. Additionally, the diversity of Al-resistant microorganisms, which are likely to play a key role in various microbially mediated processes in acidic forest soils, is low (Kunito et al., 2012a), which might result in their low functional diversity. In the present study, we investigated the effects of Al on microbial biomass and soil enzyme activities associated with carbon (C), N, and phosphorus (P) cycling in Japanese forest soils. In addition to toxicity of soluble and exchangeable Al, noncrystalline and organically bound forms of Al might limit microbial activity, because they have been reported to stabilize organic matter (Baldock and Skjemstad, 2000; Kleber et al., 2015). Hence, these Al and Fe pools, as well as soluble and exchangeable Al, were determined.

2. Materials and methods

2.1. Soils

Soil samples were collected by hand trowel from a 0–15-cm depth in the A horizon of forest soils in Nagano Prefecture, Japan: 25 soils were classified as Inceptisols (Brown Forest soils in the Japanese system), seven soils were classified as allophanic Andisols (Allophanic Kuroboku soils in the Japanese system), and eight soils were classified as nonallophanic Andisols (Nonallophanic Kuroboku soils in the Japanese system). Sampling sites were located at elevations ranging from 480 m to 2050 m a.s.l. About one-half of the collection sites were under larch plantations (*Larix kaempferi*), and the rest were under mixed broadleaf forests. The mean annual precipitation and air temperature at meteorological stations located near the sampling sites ranged from 865 to 2262 mm and from 7.1 to 11.8 °C, respectively. Each soil sample was sieved through 2-mm mesh and homogenized thoroughly. A portion of each sample was air dried for chemical analysis, whereas the remainder was maintained field moist at 4 °C until its pre-incubation for microbial analyses conducted within a year after sampling.

2.2. Chemical analyses

Soil pH was measured in distilled water or 1 M KCl with a soil-to-solution ratio of 1:2.5 (w/v). Amounts of total C and N were measured by dry combustion using an NC analyzer (MT-5, Yanaco, Kyoto, Japan). To estimate dilute salt-extractable Al, 0.01 M $CaCl_2$ was used as described by Hoyt and Nyborg (1971). According to Wright et al. (1989), 0.01 M $CaCl_2$–Al represents soluble plus some exchangeable Al. Exchangeable Al (including soluble Al) was extracted using 1 M KCl as described by Bertsch and Bloom (1996). Both $CaCl_2$–Al and KCl–Al were reported to have a close correlation with the plant Al concentration and plant yield (Hoyt and Nyborg, 1971). Sodium pyrophosphate (0.1 M) at pH 10 and 0.2 M acid ammonium oxalate at pH 3 were used to dissolve organically bound (Al_p and Fe_p) and both organically bound and noncrystalline forms (Al_o and Fe_o) of Al and Fe, respectively (Blakemore et al., 1981). The difference between the amounts of Al_o and Al_p and between the amounts of Fe_o and Fe_p provides an approximation of the amount of noncrystalline Al, including allophane and imogolite, and the amount of noncrystalline Fe, including ferrihydrite, respectively. Superfloc was used to remove colloids, and after centrifugation, supernatants were analyzed for Al and Fe with an inductively coupled plasma-mass spectrometer (7500C, Agilent Technologies, Santa Clara, CA, USA) and an atomic absorption spectrometer (AA-680, Shimadzu, Kyoto, Japan), respectively. Exchangeable Ca, Mg, K, and Na were extracted using 1 M CH_3COONH_4 (pH 7.0) and then measured by atomic absorption spectrometry as previously described (Moro et al., 2014). All subsequent data are expressed on dry weight bases.

2.3. Microbial biomass and soil enzyme activity measurements

For soil microbial biomass and enzyme activity measurements, soil samples were pre-incubated for 1 week at 22 °C after adjustment to 60% of the water holding capacity (Kunito et al., 2001). Microbial biomass C and N in the soils were measured by the chloroform fumigation-extraction method as described previously (Vance et al., 1987; Kunito et al., 1999a). Soil was fumigated with ethanol-free chloroform for 24 h at 25 °C and then extracted with 0.5 M K_2SO_4 for 30 min. The amounts of organic C in the extracts were measured with an organic carbon analyzer (TOC-V, Shimadzu), and amino acid N was measured by the ninhydrin reaction. Soil microbial biomass C was calculated as follows:

$$\text{Biomass C}\left(\mu g\ g^{-1}\right) = 2.04 \times E_c$$

where E_c = (the amount of C (μg g^{-1}) extracted from fumigated soil by 0.5 M K_2SO_4) − (the amount of C (μg g^{-1}) extracted from non-fumigated soil by 0.5 M K_2SO_4) (Inubushi, 1992). Soil microbial biomass N was calculated as follows:

$$\text{Biomass N}\left(\mu g\ g^{-1}\right) = 5.0 \times E_{NIN}$$

where E_{NIN} = (the amount of ninhydrin-reactive N (μg g^{-1}) extracted from fumigated soil by 0.5 M K_2SO_4) − (the amount of ninhydrin-reactive N (μg g^{-1}) extracted from nonfumigated soil by 0.5 M K_2SO_4) (Inubushi, 1992). These measurements were determined in triplicate for each soil.

Regarding C-acquiring enzymes, we focused on β-D-glucosidase and polyphenol oxidase in this study, because β-D-glucosidase activity is the rate-limiting enzyme in the degradation of cellulose to glucose and it is widely distributed in nature (Tabatabai, 1994; Alef and Nannipieri, 1995), and because polyphenol oxidase catalyzes the rate-limiting step in the overall decomposition of litter