Distribution and phytotoxicity of soil labile aluminum fractions and aluminum species in soil water extracts and their effects on tall fescue

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ABSTRACT

Soil acidification can alter the biogeochemistry of ecosystems and adversely affect biota; however, there are still many debates about the toxicity of aluminum (Al) fractions and Al species in soil:water extracts to plants. In this study, five crude soils with different pH values (4.92–8.51) were collected, seeded with tall fescue and grown in rhizosphere boxes for 120 days. Then, soil properties, labile Al fractions and Al species in soil:water extracts were determined, and their toxicities to plants were analyzed. Our study showed that a stable exchangeable Al fraction (ExAl) pool exists and is supplied by other labile Al fractions. Dissolution of Al from adsorbed hydroxyl-Al fraction (HyAl) and organic-Al fraction (OrAl) may play important roles in soil Al toxicity, as HyAl and OrAl account for major parts of soil labile Al. Additionally, Al^{3+} and mononuclear hydroxyl-Al species in soil:water extracts have few effects to plants. Nevertheless, high negative correlations were found between Al-F$^-$ complexes and tall fescue biomass, indicating their toxicity in the natural soil environment. Thus, in many cases, Al^{3+} toxicity should not be emphasized because of its lower activity in soil water extracts. Moreover, toxicities of $AlF_3(aq)$ and AlF_4^- to plants should be emphasized, because they have been confirmed in soil water extracts in this study.

1. Introduction

About one third of the total land area on the planet consists of acid soils, and as much as half of the potentially arable lands are acidic (Kochian et al., 2004). Soil acidification is also a serious problem in China, and it has been reported that soil pH decreased significantly in the last two decades of the twentieth century in major Chinese crop-production areas (Guo et al., 2010). Aluminum (Al) is a strongly hydrolyzing metal and its chemistry is determined by pH (Brautigan et al., 2012). The solubility of Al is enhanced under acidic conditions or in the presence of certain complexing ligands (Brautigan et al., 2012). Many plant species are sensitive to Al toxicity at low micromolar concentrations of Al in hydroponics solutions, therefore, there is great potential for Al toxicity in soils (Delhaize and Ryan, 1995). Moreover, regions with large amounts of rainfall tend toward greater acidity and release

more Al from the dissolution of solid Al fractions, leading to higher toxicity to plants (vanPraag et al., 1997; von Uexküll and Mutert, 1995).

Large amounts of Al inhibit root growth, damage root tissues and cause deficiencies in Ca, Mg and P as well as other physiological stresses (Bakker et al., 1999; Collignon et al., 2012). However, different Al fractions in soil have different physical-chemical reactivity and bioavailability, which affect soil properties and the toxicity of Al to plants (Drabek et al., 2005). In this case, the total amount of Al in soils is not always significant. Selective dissolution methods (for example, extracts of 1 M KCl, pyrophosphate, acid ammonium oxalate, dithionite–citrate–bicarbonate (DCB), and 0.5 M NaOH) are widely used to quantify Al fractions for soil classification or to characterize specific soil components (Garcia-Rodeja et al., 2004). Studies on Al sequential extraction have been brought to our attention in recent decades, and to date,

labile Al sequential procedures usually employ KCl, pyrophosphate, and acid ammonium oxalate extracts to distinguish exchangeable Al (ExAl), organic-Al (OrAl) and hydroxyl-Al (HyAl) (Soon, 1993). However, the sensitivity of pyrophosphate extracts asks for strict experimental conditions and it may reflect uncertainties in the sources of this fraction (Paterson et al., 1993). For example, pyrophosphate may also extract some interlayer Al and relatively labile forms of surface-precipitated Al (Garcia-Rodeja et al., 2004). Thus, the extraction of HyAl has been a priority instead of OrAl in these years (Garcia-Rodeja et al., 2004; Shao et al., 1998).

In acid soils, Al toxicity to plants is more closely related to the activity of Al^{3+} in soil solution than to solid-phase properties (Menzies et al., 1994a). With the help of simulation software, such as Geochem-EZ and Visual Minteq, more and more studies of Al species in soil:water extracts have improved our understanding of Al toxicity (Alvarez et al., 2011; Brown et al., 2008; Yan et al., 2013). However, the toxicity of Al species in hydroponic solution or soil:water extracts to plants remain unclear. There are still many debates about the toxicity of mononuclear hydroxyl-Al species and Al-F⁻ complexes (Kinraide and Parker, 1989; Peregrina et al., 2007; Stevens et al., 1997). Moreover, there are still few studies concerned with the speciation of Al in the soil:water extracts of crude rhizosphere soil and their toxicity to plants.

The aim of this research was to illustrate the toxicity of Al fractions and Al species of crude soil in tall fescue (*Festuca arundinacea Schreb*), which is a major cool-season forage grass grown in the temperate regions of the world and shows remarkable potential for heavy-metal phytoremediation (Chen et al., 2017; Huang et al., 2017; Zhuo et al., 2017). In this case, five crude soils with different pH were collected from Lichuan City and placed in rhizosphere boxes. The boxes were planted with tall fescue seeds that were allowed to grow for 120 days. Then, the soil properties, solid labile Al fractions and Al species in 1:1 soil:water extracts of rhizosphere soil were determined. In addition, the toxicities of Al fractions and Al species on tall fescue were carefully analyzed.

2. Materials and methods

2.1. Soil sites

Surface soil samples (0–15 cm) were collected from Lichuan City, located in Enshi prefecture (Hubei province, P.R. China), which has a monsoon-influenced humid subtropical climate: short, cool winters; hot, humid summers; and high humidity year-round (https://en.wikipedia.org/wiki/Enshi_City). Five crude soil samples were collected from Shibanling village (SBL), Jingui village (JG), Longfeng village (LF), Shimiaozi village (SMZ) and Xuetangping village (XTP) as shown in Fig. S1. Soils were air-dried and sieved using 2-mm mesh sieves and part of the soil (BS, bulk soil) was collected for analysis and the rest of the soil was loaded into rhizosphere boxes as described in Fig. S2.

2.2. Plant seeding and soil sampling

Before cultivation, a commercial type of tall fescue (houndog 5) was initially seeded in plastic pots (7.5 cm in diameter and 8.5 cm deep) in a perlite-peat (1:1, v/v) medium. After germination, tall fescue seedlings were kept in a greenhouse with a daily maximum/minimum temperature of 24/20 °C for a 16 h photoperiod (300 µmol photons $m^{-2} s^{-1}$ PAR) and watered with 1/2 Hoagland solution for 40 days, allowing the roots and shoots to establish (Chen et al., 2013). Next, the seedlings were transplanted into rhizosphere boxes and kept in the greenhouse for another 120 days; during this period, the seedlings were watered with deionized water every three days. Then, the rhizosphere boxes were disassembled and near-rhizosphere soils (NR), far-rhizosphere soils (FR) and tall fescue were harvested for the following studies.

2.3. Soil analysis

The soil texture of the bulk soil was measured by laser-diffractometry (Vancampenhout et al., 2010), and the soils were initially digested in H_2O_2 to remove organic matter and then dispersed by 0.5 mol/L NaOH. The soil pH was obtained from 1:2.5 soil:water extracts. The cation exchangeable capacities (CEC) of the acid soils were determined by saturating the soils with 1 M NH_4OAc at pH 7 followed by distillation and titration (USDA-NRCS, 2004) (4B1a1a1a1). To determine the CEC of calcareous soils, 1 mol/L NH_4Cl was used to remove calcium from the soil; next, the samples were washed and the method for CEC determination given above was used. The saturating solution 1 M NH_4OAc was collected, and the K^+, Ca^{2+}, Na^+, Mg^{2+} and Al^{3+} concentrations in the saturating solution were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Avio200, PerkinElmer Corp., Norwalk, CT). The exchangeable base content was calculated as the sum of K^+, Ca^{2+}, Na^+ and Mg^{2+}. Total nitrogen (TN) was measured with the Kjeldahl method (USDA-NRCS, 2004, 6B3a), which consists of titration after distillation and sample digestion. Available N (alkali-hydrolyzable nitrogen) was released and transformed into NH_3 by 1.8 mol/L NaOH and Zn-FeSO₄ powder (1:5, w/w) at 40 °C for 24 h and then absorbed with 2% H_3BO_3 before titrating with 0.005 mol/L H_2SO_4 (Xiong et al., 2008). Total carbon (TC) and total organic carbon (TOC) contents were measured by the combustion oxidation nondispersive infrared absorption method with a multi N/C 3100 (Analytikjena, Germany), in which the TC of soils can be measured directly by the equipment and the TOC of the soils can be measured after the removal of inorganic carbon by 5% phosphate acid. The available P (AP) contents of acid and calcareous soils were extracted by Bray-1 solution (0.03 mol/L NH_4F, 0.025 mol/L HCl) (Xiong et al., 2008) and Olsen solution (0.5 mol/L $NaHCO_3$, pH 8.5) (Sun et al., 2015), respectively, and were determined colorimetrically with ammonium molybdate. The total amount of Al in bulk soil was quantified using ICP-AES after digestion with a mixture of HNO_3, HF and H_2O_2 (3:1:1, v/v/v). Additionally, standard reference soils (GBW07458, GBW07460) were studied through soil analysis as a part of soil quality control.

2.4. Solid labile Al fractions

Sequential extraction of solid labile soil Al followed the methods introduced by Shao et al. (1998), in which the extraction of adsorbed HyAl was completed by 0.2 mol/L HCl due to the limitations of acid ammonium oxalate techniques (Garcia-Rodeja et al., 2004). Briefly, ExAl, HyAl and OrAl were extracted sequentially by 1 mol/L KCl, 0.2 mol/L HCl and 0.1 mol/L $Na_4P_2O_7$ (pH 10). For extraction with 1 mol/L KCl, 5 g of soil was shaken for 2 h with 50 mL extraction solution, and the supernatant was collected after 15 min of centrifugation at 3000 rpm. The procedure was repeated, the supernatant was combined, and the Al content was determined by ICP-AES after filtering. The remaining soil was washed with deionized water and then used to extract HyAl. For extraction with 0.2 mol/L HCl, the remaining soil was shaken for 2 h with 50 mL of the extraction solution and then the procedure was repeated. Then, the supernatant solutions were combined, and the Al content was determined by ICP-AES after filtering. For extraction with 0.1 mol/L $Na_4P_2O_7$ (pH 10), the remaining soil from the HyAl extraction was shaken for 2 h with 100 mL of the extraction solution, and the supernatant solution was collected after 15 min of centrifugation at 3000 rpm. This procedure was repeated, the supernatant solutions were combined, and the Al content was determined by ICP-AES after filtering.

2.5. Al species in 1:1 soil:water extracts

Soil pH and electrical conductivity (EC) were measured immediately in 1:1 soil:water extracts (Brown et al., 2008). The ionic