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Plant functional groups, grasses versus forbs, differ in their impact on soil carbon dynamics with nitrogen fertilization

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ABSTRACT

Nitrogen (N) addition in N-limited grasslands often increases aboveground productivity, decreases species richness and leads to changes in species composition. In contrast to these consistant results in aboveground vegetation parameters, there is no consistant pattern in how N fertilization affects soil organic carbon (SOC) dynamics. Our objectives were to test how plant functional group changes caused by N fertilization affect soil C dynamics and determine if different plant functional groups respond similarly. We conducted a two-factorial experiment to examine soil C dynamics with N fertilization and soil inoculation with field microbial communities in a greenhouse pot experiment. We used six plant species (two grasses and four forbs) that are dominant within sub-alpine meadows on the east part of the Qinghai-Tibetan plateau. For both grasses and forbs, N fertilization and soil inoculation, alone or in combination, decreased SOC by 4–10% and increased soil microbial biomass. For grasses, N fertilization combined with inoculation caused much lower SOC content and higher soil microbial biomass carbon (MBC) as compared to inoculation alone. In contrast to forbs, grass aboveground biomass was significantly negatively correlated with SOC change and positively correlated with MBC change. Nitrogen fertilization combined with inoculation significantly increased basal respiration and cumulative C mineralization rates for both grasses and three of the four forbs as compared to inoculation alone. Grasses had higher basal respiration rates than forbs under these two treatments. Despite higher aboveground grass biomass, N-fertilization lowered the SOC pool by increasing soil MBC and basal respiration rates, thus increasing C decomposition. Overall, in these sub-alpine meadows, grasses and forbs impact on soil C dynamics differs and grasses, but not forbs, may reduce soil C sequestration in response to N fertilization.

1. Introduction

Many terrestrial ecosystems are N-limited [1], and N addition can dramatically increase productivity, decrease species richness and lead to changes in plant species composition [2–4]. However, the effects of N addition on soil organic carbon (SOC) dynamics and soil C sequestration differ between sites [4–8]. Some studies have reported that N fertilization can increase plant productivity and soil C sequestration [8–10] because of decreased SOC decomposition [11–14], linked with a decrease in microbial biomass and activity. Other studies have shown that N fertilization-induced increases in plant productivity do not necessarily result in increased soil C accrual [5,8]. A negative effect of N fertilizers on soil C sequestration may result from increased SOC decomposition caused by a "priming effect", i.e. accelerated SOC decomposition caused by increased microbial biomass and/or activity because N-fertilization stimulates plant growth and thereby increases rhizodepositions and other plant residue inputs [15,16].

Grasslands may differ in plant diversity and dominance of functional groups, and their subsequent impact on soil C sequestration among sites [4,17]. High plant species richness can increase soil C sequestration [17,18]. However, such increase can largely be attributed to the presence and abundance of specific species or functional groups [17,19], rather than to species richness per se. For instance, in tallgrass prairie grasslands in North America, C_4 grasses and legumes increased soil C accumulation by 193% and 522%,

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respectively [17]. Within alpine meadows, litter quality (e.g. N content and C:N) rather than litter quantity has been shown to drive soil C sequestration [20]. Plant species identity also can affect the soil microbial community size, composition and activity which in turn can influence grassland functions such as soil C storage [19,21].

Sub-alpine meadow is one of the dominant grassland types present in the Qinghai-Tibetan Plateau and because of its high SOC content may play an important role in global warming [22,23]. Nitrogen fertilization may decrease SOC [4,24]. Nitrogen fertilization at rates of 5, 10 and 15 g m^{-2} yr^{-1} decreased surface layer soil C accumulation by altering vegetation composition, i.e. increased grass dominance and decreased legume and forb biomass [4,25], which also caused a lower plant tissue C:N ratio [4]. However, little is known about how these plant functional groups influence soil C dynamics in response to N fertilization.

We conducted a short term experiment with six plant species that are common in sub-alpine meadows to examine how different plant species from different functional groups affect soil C dynamics with N fertilization. We used sterilized soil inoculated with field microbial communities to examine the potential of plant C exudates to "prime" microbial growth and/or activity and determine soil C sequestration. We hypothesized, based on previous fertilization studies [5,8,15,16], that N fertilization and soil inoculation, alone or in combination, increase microbial biomass carbon (MBC), microbial respiration and C mineralization rates and as a consequence decrease SOC. And we hypothesized that functional groups differ in their effect on soil microbial biomass and activity, and thereby affect soil C storage and sequestration in sub-alpine meadows [19,21].

2. Material and methods

2.1. Site description

Soil for both the pot experiment and inoculum was collected from the surface layer 15 cm of a sub-alpine meadow with high plant diversity (plant species richness of 40–50 per 0.25 m^2) [25] at the Research Station of the Alpine Meadow Ecosystem of Lanzhou University, located in Hezuo, Gansu, eastern Qinghai-Tibetan Plateau of China (N34°55′, E102°53′, 2900 m above sea level). Hezuo has a 30-year mean annual precipitation of 550 mm, with 85% of the precipitation occurring during the growing season from June to September (Institute of Hezuo Meteorology). The mean annual temperature is 2.4 °C, ranging from −8.3 °C during December–February to 11.9 °C during June–August periods. The soils in this area are chestnut soils or Haplic Calcisols according to the FAO classification or sub-alpine meadow soils according to the Chinese Soil Classification System [26]. The soil texture is 20% sand, 60% silt and 20% clay.

The sub-alpine meadow is dominated by perennial sedge *Kobresia humilis* Serg (Cyperaeae). Common species include grasses, such as *Stipa aliena*, *Elymus nutans* Griseb, *Festuca sinensis* Keng ex S. L. Lu, forbs, such as *Saussurea superba* Anth., *Gentiana lawrencei* Burk. var farreri T.N.Ho, *Gentiana straminea* Maxim., *Potentilla nivea* Linn., *Potentilla fragarioides* L., *Scirpus distigmaticus* Tang et Wang; and Cyperaeae sedges such as *Kobresia pygmaea* C.B. Clarke in Hook, and *Carex* sp.

2.2. Preparation of soils and inoculum

To examine the potential of plant C to "prime" microorganisms and their subsequent effect on soil C sequestration, soils collected from the field were sterilized and then inoculated with the soil microorganisms from the same sub-alpine meadow. Soils taken from the top 15 cm (Ah horizon) of the sub-alpine meadow were sieved to 6 mm to remove stones and large plant particles, homogenized, and sterilized by γ-irradiation (25 kGy). Soil MBC was measured before and after sterilization. Soil MBC after sterilization was 0.138 g kg^{-1}, which was less than 5% of the mass prior to sterilization. The SOC content averaged 30.72 g kg^{-1}.

The soil sample used as inoculum was collected from the top 15 cm of the same sub-alpine meadow 3 days before the inoculation treatments. The microbial inoculum was made by adding 15 L of sterile MilliQ-filtered water to 15 kg fresh soil [27]. These mixtures were left for 5 h to enable large particles to sink, after which the supernatant was sieved through a 75 μm mesh, followed by two sievings through a 45 μm mesh and two sievings through a 30 μm mesh. This method omitted the micro-arthropods and nematodes but allowed most other microorganisms in the suspension to pass through [28].

2.3. Pot experiment

The pot experiment was carried out in a greenhouse with an average 16/8 h day/night cycle and a light:dark temperature regime of 20/12 °C. Temperature conditions were regulated by computerized control of heating, vents, screens and a mobile air conditioning unit. The light regime was minimally 16 h/d of light, and natural daylight was supplemented with metal halide lamps (225 μmol m^{-2} s^{-1} photosynthetically active radiation) to ensure optimum light supply. Before transplanting seedling, 11 cm high pots with an inner diameter of 9–13 cm (base to top), sterilized with alcohol, were filled with 900 g γ-irradiation sterilized soil and cultured for thirty days in the greenhouse. Soils were watered to field capacity every six days to ensure them not too loose.

Seeds of six individual species (grasses *Festuca sinensis* Keng ex S. L. Lu, *Elymus nutans* Griseb, and forbs *Lamium amplexicaule* Linn., *Aconitum gymnandrum* Maxim, *Elsholtzia densa* Benth., and *Potentilla fragarioides* L.), collected at the field site, were sterilized with a 0.1% chloride solution for 3 min and sown on glass beads, moistened with demineralized water. For germination seeds were placed in a cabinet with a 16:8 h light:dark photo regime and an 18:10 °C temperature regime. Prior to this procedure, a seed germination pre-experiment was carried out. Based on the differences among the species, sowing started at different times to ensure all species germinated at the same time and were of a comparable ontogenetic state at the start of the experiment. One week after germination, the seedlings were transplanted. Six seedlings of the same species were planted per pot. The pots were randomly divided among four treatments (four replicates per treatment): N fertilization and soil inoculation (N-M), N fertilization alone (N-O), soil inoculation alone (O-M), and a control with neither N fertilization nor soil inoculation (O-O). All pots were randomly placed on trolleys in a greenhouse. The positions of the trolleys were changed three times a week to minimize effects of microclimate differences within the greenhouse.

Once planted, the N-M and O-M pots were injected with a total of 145 ml microbial inoculum originating from the field soil community or, as a control (N-O and O-O), 145 ml of sterile MilliQ-filtered water at the first, second, and third day after transplanting. A week later, the N-addition treatments started: pots were watered 5 days a week for 9 weeks, each time with 25 ml of either sterile MilliQ-filtered water (no N addition; O-M and O-O pots) or NH_4NO_3 solution (N addition; N-M and N-O pots), at a rate of 2.1 g N m^{-2} yr^{-1}. The approximate growing season in the Tibetan Plateau is about 5 months, and the N addition rate is comparable to the fertilization rate in previous field N fertilization experiments in the Tibetan Plateau [4,25]. We harvested the aboveground biomass and roots of all plants 10 weeks after planting. Aboveground