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# Substrate-induced respiration responses to nitrogen and/or phosphorus additions in soils from different climatic and land use conditions



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#### ABSTRACT

The fates of carbon (C) input to soils widely vary under different nutrient availability. In this study, both initial substrate-induced respiration (SIR) rates and substrate-induced growth responses to nitrogen (N) and/or phosphorus (P) additions were investigated together because the two approaches had been assessed separately in previous studies. A wide range of Japanese, Thai and Kazakh soils with different land uses were used, including acidic Japanese forest soils with low N availability, which has not been fully examined. We hypothesized that microbes in Japanese forest soils would mainly respond to N addition. The results showed that N addition decreased initial SIR rates to 75–87% for the acidic Japanese and Thai forest soils probably due to the increases in substrate C use efficiencies, but had no effect for other soils. P addition tended to increase initial SIR rates. In addition, P but not N addition resulted in the increases of specific microbial growth rates for all tested soils. In conclusion, soil microbes responded to P addition with enhanced their growth rates and initial SIR rates, whereas they responded to N addition with reduced initial SIR rates probably through efficient use of C only for the Japanese and Thai forest soils. Therefore, nitrogen availability will have a crucial role in microbial use of substrate C especially for acidic forest soils with low N availability.

#### 1. Introduction

Carbon (C) inputs to soils fuel much of the belowground heterotrophic microbial activity [1], since C is often considered to be the primary factor limiting soil microbial activity [2]. C provides both a source of C resulting in the production of new cells (assimilation) and a source of energy resulting in the formation of CO<sub>2</sub> (respiration) in an aerobic condition [3]. Nitrogen (N) is considered to be the major limiting nutrient to net primary production in most terrestrial ecosystems [4]. In contrast, the N limitation may be alleviated by the inputs of N from anthropogenic sources, such as N fertilizer, human waste products, and atmospheric pollutants, especially near areas of high human activity [5]. Therefore, understanding microbial nutrient (especially N) limitation and the fate of the C inputs and their interaction with nutrients is fundamental for predicting future changes in soil C storage [1].

The commonly employed approach to assess both N and phosphorus (P) limitations in soil microbes is to evaluate substrate-induced growth responses (SIGR) to the N and/or P additions which involves measuring

soil microbial respiration during  $\sim 3$  days after the addition of a sufficient concentration of glucose C as a model substrate of the labile C input in combination with N and/or P to soils [2,6,7]. However, this approach is limited because nutrient limitation is induced by the addition of high concentrations of glucose C during a relatively long incubation time [8]. In contrast, the responses of respiration rates within a few hours after the addition of glucose (i.e., initial substrate-induced respiration (SIR) rates) to N and/or P additions will reflect in situ nutrient availability in soils and, therefore, are expected to be a sensitive indicator of microbial nutrient limitation, because the initial SIR responses are not affected by the changes in nutrient status induced by the increases in microbial biomass. In addition, the results of this approach will be used to predict the fate of C inputs under different nutrient conditions. Dilly [6] found that initial SIR responses decreased when N and P were added, and this is probably because glucose C use efficiencies, defined as the ratio of assimilation over C uptake (i.e., the sum of assimilation and respiration), would increase due to alleviation of the nutrition limitation in some originally nutrient-limited soils. Blagodatskaya et al. [9] also found that initial respiration rates after the

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glucose addition with N were lower than those without N in an unfertilized soil. Recently, Poeplau et al. [10] also found the reducing effect of the N but not P addition on initial SIR rates only in N-deficient soils of long-term agricultural experiments with different fertilization treatments. Therefore, the initial SIR responses to the N and P additions may provide vital information on nutritional conditions in different soils. However, to our knowledge, initial SIR rates and SIGR to N and/or P additions had been assessed separately in previous studies. In addition, there have been insufficient studies using a wide range of soils from different climatic and land use conditions.

Poeplau et al. [10] concluded that short-term nutrient effects might be more important for nutrient-poor but C-rich ecosystems, which would be particularly true for forest ecosystems. In Asia, we can find various types of soils with a large range of nutrient conditions [11]. In particular, in Japanese forest soils under a climate where the average annual precipitation is about 1700 mm, which is about twice the global average, the level of available N is lowered with high annual precipitation which will govern the degree of leaching [12]. Therefore, we hypothesized that microbial respiration after glucose addition would be more sensitive to N addition in Japanese forest soils with lower levels of available N.

The objective of this study was to compare the initial SIR rates and SIGR to N and/or P additions for a wide range of Asian soils collected from different climatic and land use sites, including Japanese forest soils with low N availability.

#### 2. Materials and methods

#### 2.1. Soils

Soil samples were collected from 13 sites under different climatic and land use conditions in Japan, Thailand, and Kazakhstan as several case studies. Typical soils were selected in each country, and selected soils were known to cover wide ranges of soil properties, especially annual precipitation totals and soil pH [11]. In Japan, two forest soils were collected from a Japanese hinoki cypress (Chamaecyparis obtusa) developed on sedimentary rocks and volcanogenic materials as parent materials in Kochi Prefecture (N33°26', E133°1' and N33°28', E133°0', respectively) [12]. The soils were classified as a Dystric Cambisol and an Umbric Silandic Andosol according to the World Reference Base for Soil Resources [13], and thus hereafter referred to as Cambisol-f1 and Andosol-f1, respectively, and f indicates forest. Two forest soils were collected from a deciduous forest, predominantly beech (Fagus crenata) and oak (Quercus mongolica) in the Tango Peninsula, Kyoto Prefecture (N35°37', E135°10'). The soils were classified as an Entic Podzol and a Dystric Aluandic Andosol [13], and referred to as Podzol-f and Andosolf2, respectively. Forest soil was collected from a mixed deciduous and evergreen forest (Quercus serrata and Shiia cuspidata) in the city of Kyoto in Kyoto Prefecture (N35°01', E135°47'), classified as a Dystric Cambisol [13], and referred to as Cambisol-f2. Two arable soils were collected from the cropland and paddy fields in the Field Museum Hommachi, Field Science Center, Tokyo University of Agriculture and Technology, Tokyo Prefecture (N35°40', E139°28'). Both soils were classified as Hydragric Anthrosols [13], and referred to as Anthrosol-c1 and Anthrosol-p, and c and p indicate cropland and paddy, respectively. Arable soil was collected from the Chinese cabbage field on the Experimental Farm of Kyoto University, Osaka Prefecture (N34°51', E135°38') [14], classified as a Hydragric Anthrosol [13], and referred to as Anthrosol-c2. In Thailand, three soils were collected in the village of Du La Poe, Mae Hong Son Province, Northern Thailand (N18°24', E98°05'), where a traditional style of shifting cultivation is still used [15]. The forest soil was collected from natural forests, classified as a Chromic Acrisol [13], and referred to as Acrisol-f. The arable soil was collected from a cropland rice field that had been cultivated for 2 months following slash and burn agricultural practices. The fallow soil was collected from a fallow bush field at 2 years after cultivation with a

slash and burn technique. Both soils were classified as Dystric Chromic Leptic Cambisols [13], and referred to as Cambisol-c and Cambisol-b, and c and b indicate cropland and fallow bush, respectively. In Kazakhstan, two soils were collected from a study site located in the northern foothills of the Tian Shan Mountains (locally known as the Ketmen Mountains) east of Almaty (43°11′N, 79°27′E). The forest and grassland soils were collected from a coniferous forest (*Picea schrenkiana*) and natural grassland, respectively, where the coniferous forest was scattered in natural grassland vegetation. The forest soil was classified as a Leptic Umbrisol [13], and referred to as Umbrisol-f. The grassland soil was classified as a Cambic Umbrisol [13], and referred to as Umbrisol-g, and g indicates grassland.

The sites in Japan have humid climates, while those in Thailand have a distinct dry season, and those in Kazakhstan have a drier climate than the other sites. In all sites, the annual potential evapotranspiration does not exceed annual precipitation totals (Table 1). The soil moisture regimes are udic in Japan and ustic in Thailand and Kazakhstan. The soils are derived from non-calcareous parent materials. Therefore, the climate conditions, especially the amounts of annual precipitation, influence the soil nutrient availability and pH for the natural forest and grassland soils because those will govern the degree of leaching [11]. In addition, the forest and grassland soils are expected to have received less N input from anthropogenic sources than the arable and fallow soils. In contrast, the arable and fallow soils are expected to have high N availability due to the effects of land management such as fertilization in Japan and ash input from burning in Thailand which have stimulated mineralization activities of soil microbes [15].

Mineral soil samples were collected from 4 to 6 points selected at random in each site to a depth of 10 cm after the removal of plant debris and organic horizons, then pooled to form a composite sample for each site. Freshly collected soil samples were sieved through a 2 mm sieve, homogenized, and stored at field moisture and 5 °C until use. Subsamples of the soils were air-dried and then analyzed for soil pH, organic C, total N, and K<sub>2</sub>SO<sub>4</sub>-extractable C and N as previously described [16] (Table 1).

The freshly collected soil samples were pre-incubated in Erlenmeyer flasks for 1 week at 25 °C to reduce the influence of disturbance resulting from collection and sieving. The moisture contents for the main incubation experiment were adjusted to 50–55% of the maximum water holding capacity (WHC) for all soils except for the Anthrosol-c2 soil (35%). The moisture content for the Anthrosol-c2 soil was chosen to obtain maximum SIR rates based on a preliminary experiment in which the SIR rates were determined at various moisture contents (25, 35, 45, 55, and 60% of the maximum WHC) [16]. The moisture contents for pre-incubation were set to a value lower than those for the main incubation (i.e., about 45–50% (but 30% for the Anthrosol-c2 soil) of the maximum WHC), since the pre-incubated soils were further treated with a substrate solution.

#### 2.2. Substrate-induced respiration responses to N and/or P additions

Initial SIR rates and SIGR to N and/or P additions were investigated to assess the impacts of N and P availabilities on soil respiration rates after the addition of glucose as a model substrate of the C inputs. Following pre-incubation, each moist soil sample was divided into aliquots (10 g of oven-dried soil) in plastic cups. Each soil sample (with three replicates) was amended with a substrate solution containing glucose and/or mineral N and/or P at the following concentrations to a final moisture content of about 50–55% (35% for the Anthrosol-c2 soil) of the maximum WHC. The substrate solution contained (1) glucose at a rate of 4000  $\mu$ g C g<sup>-1</sup> soil (+C); (2) glucose, N as NH<sub>4</sub>NO<sub>3</sub>, and P as KH<sub>2</sub>PO<sub>4</sub> at a rate of 400 and 400  $\mu$ g N and P g<sup>-1</sup> soil (+CNP); (3) glucose and N (+CN); or (4) glucose and P (+CP). To avoid nutrient deficiency, sufficient amounts of N and P were supplemented at a final concentration of 0.1 g N or P per g C added to the soil as glucose. We did not test the +CN and +CP treatments for the Cambisol-f2,

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