The relationship between soil bacteria carbon utilization and soil physicochemical properties

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Abstract. This study aimed to explore the relationship between carbon utilization pattern of soil bacterial communities and the soil physicochemical properties in temperate forest of Poland under two different climatic conditions. Ten different forest soils from three forest types (deciduous, mixed, and coniferous forest) were incubated in laboratory conditions in two temperatures: 10°C and 30 °C, through 8 months. The soil bacteria carbon utilization was investigated using Biolog® Eco plates. The number of carbon substrate decayed on Biolog® Eco plates declined after the incubation in all studied forest soils. The soil samples originating from deciduous forest which were highly distinct performance with others correlated to their soil properties which were also highly different. Several soil properties e.g. pH, C/N ratio, SOM and the relation with vegetation types were found affecting the carbon utilization by bacterial communities. However, in this study, the relation between soil bacterial communities carbon utilization with soil physicochemical properties is limited, as soil bacteria are characterized by the high ability to adapt.

1. Introduction

Soil bacteria as crucial drivers of nutrient turnover in terrestrial ecosystems has been long recognized about their involvement in the key processes of soil, such as organic matter degradation, mineralization, and humification [1–3]. The heterotrophic bacteria use organic carbons and mineralize part of them into CO_2, contributing the flux of global CO_2 in the atmosphere. The bacteria fix CO_2 in the atmosphere, then mediated by the allocation of recalcitrant or simple SOM into the soils. When the SOM decomposed and used by bacteria to build their biomass, and at some point necromass which stabilized in soils [4–6].

Numerous factors are influencing the bacterial community activity on degrading carbon sources. Some studies emphasized the role of climatic factors as driving elements for microbial decomposition, whereas other studies stressed the role of substrate quality [7–9]. A study suggested that the bacterial communities show a stronger response to changes in soil properties rather than vegetation composition [10]. Meanwhile, a study of bacterial communities related to particular factors is more likely irrelevant with the real condition in the field, thus, the study combining climatic factors, soil properties, and vegetation types is needed.

Temperature has also been known as important environmental factors governing nutrient availability and bacteria activity [11,12]**.** It has been considered to be the main factor influencing the decomposition, and as a key limiting factor for bacterial metabolisms [13–15]. The changing of soil temperature may give an impact on bacterial community to alter their carbon utilization strategies in order to adapt to changing temperature [16]. Other soil physicochemical properties such as pH, SOM, C/N ratio and relation with litter types have also been recognized as key limiting factors for soil bacteria. Some previous studies demonstrated that soil properties especially pH have a strong influence on overall composition of bacterial communities [10,17–20]. The indirect relation between litters and pH is that in temperate forests, the litter of connifer appears to be the most acidifyng, followed by beech, oak and birch. Maple horbeam, ash and lime comprised the group with least acidifying forest tree [21].

The activity of soil bacterial community also can be determined by temperature sensitivity of the decomposers, the availability of substrate, interaction with above ground processes, potential adaptations of microbial physiology, and also environmental drivers such as soil physicochemical factors [22–26]. Unfortunately, most studies related to soil bacterial communities have been focused solely on relationship with C/N ratio, moisture and temperature [27] and the relationship vegetation types [28,29]. Thus, the study of relationship between soil bacteria carbon utilization and soil physicochemical properties in relation with climatic factor and vegetation type is needed to explain broader view about soil bacteria activity on carbon utilization.

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Studying soil microorganisms may face many limitations. One of common methods to study soil bacteria metabolic profiles is using Biolog® Eco plates, that work based on measurement of colorimetric on various carbon substrate utilization. This study aimed to assess the relationship between soil bacteria carbon utilization and soil physicochemical properties differing in three types of temperate forest and under the influence of two climatic conditions.

2. Materials and Methods

2.1. Study sites and soil sampling

The study was conducted based on soil samples collected in Małopolska region (southern Poland). Three types of temperate forests were investigated: coniferous forest dominated by Scots pine (*Pinus sylvestris*), deciduous forest dominated by birch (*Betula pendula*) and beech (*Fagus sylvatica*), and mixed forest dominated with hornbeam (*Carpinus betulus*), larch (*Larix decidua*), beech (*Fagus sylvatica*), Scots pine (*Pinus sylvestris*), fir (*Albies alba*) and spruce (*Picea albies*).

The soil sample were collected during spring (April and May 2019). The top soils O horizon (0-10 or more cm depth) below plant litter layer were collected. One mixed soil sample composed of 5 soil subsamples was collected from each study sites. After collecting soil samples, they were transported to the laboratory in plastic bags, then they were sieved through 1-cm sieve to remove the green parts of plants, roots and some materials such as stone. For preincubation, soil samples were stored at 4 ˚C before embarking all analyses.

2.2. Soil physicochemical analysis

The soil moisture and organic matter content were determined by oven drying and then determining the loss on ignition [30]. Soil samples after pre-incubation were put in the glass beaker and weighed. The water content was determined by drying them at 105 ˚C for 24 hours. The soil organic matter (SOM) content was determined by calculating loss of ignition at 550 ˚C for 24 hours. Water-holding capacity (WHC) was measured by a standard gravimetric method, used for adjusting soil moisture content at 70% and for further proportion of soil and water sample. The soil pH was measured in 3 grams air-dried soils shaken in 30 mL of distilled water (1:10 ratio w/v) for 1 hour at 200 rpm. The total C and N contents were analysed by dry combustion with elemental analyser Vario ELIII Elementar Analysensysteme GmbH.

2.3. Laboratory incubation

Each soil sample of 50 grams was incubated in two temperatures (10 ˚C and 30 ˚C) through 8 months in the climatic chamber. The moisture of soil samples was adjusted to 70% of WHC. During the incubation, the soil samples were added by distilled water until reach the initial weight, and allowed the aeration by opening the jars of soil sample. The analyses of soil bacteria and soil physicochemical properties were conducted before and after incubation.

2.4. Biolog® Eco plates

The assessment of soil bacteria carbon substrate utilization was conducted using the Biolog® Eco plates. Biolog® Eco plates contained 96 wells, measuring the metabolism of 31 carbon sources in three replications. Carbon compounds are degradable by different soil bacteria. The carbon sources utilization rate was shown by changing of colourless into purple as reduction of tetrazolium violet redox dye, if inoculated bacteria utilized the substrate [31]. The six groups of carbon sources: carbohydrates, carboxilyc and ketonic acids, amines and amides, amino acids, and polymers, according to Weber and Legge [32].

The samples of each soil were shaken in 0.9% NaCl for 30 minutes (200 rounds/minutes), then allowed to settle for 30 minutes. The suspension containing microbes (100 μl) were diluted in 9.9 mL of 0.9% NaCl. The solutions were inoculated into Biolog® Eco plates (100 μl per well). The measurement was carried out in substrate utilization as light absorbance at 590 nm using Tecan spectrophotometer with i-control software. The carbon utilization of soil bacteria was calculated as substrate richness (R_s). R_s was expressed as the total number of utilized substrate in each plate. The R_s was calculated from the measurement at 96 hours of incubation.

2.5. Statistical analyses

The substrate richness (R_s) were investigated by two-way analysis of variance (ANOVA) with Tukey´s test ($p<0.05$) using Statistica 64 software version 13.3. The physicochemical properties (SOM, WHC, pH in water and KCl, and C/N ratio) in relation with the bacterial communities were analysed using Origin® Pro 2016 software.

3. Results and discussion

Biolog® Eco plates which contain 31 different carbon substrates provided organic carbon similar to the soils that were readily can be used by bacterial communities. The use of carbon substrate on Biolog® Eco plates could be observed by changing of colour from clear to violet. In our study, the soil bacteria carbon utilization assessed by Biolog® Eco plates represented by substrate richness (R_s) or number of degraded substrate differed between forest types and incubation temperature ($F_{18,60} = 12.73$, $p < 0.0001$). We also

observed that 8 months incubation in two climatic conditions affected the soil bacteria carbon utilization in deciduous forest sites and mixed temperate forest sites, while coniferous forest sites stated in higher carbon utilization. Although soil bacteria carbon utilization varied in different incubation and study sites, certain general trends were visible, where 8 months incubation at 30 °C seemed to give a negative effect on carbon utilization of bacterial communities, especially in deciduous forest. These may suggest that deciduous forest soils are more sensitive to temperature increase. Thus, during the climatic warming, the soils under deciduous forest may decompose faster and emit more CO_2 than other forest types.

The significant interaction between these two parameters (Fig. 1) indicated that the carbon utilization was higher before the incubation related to the substrate availability that support high bacterial activity. It is also suggested from prior study that the warming induced changes in substrate composition and availability [33], that cause the lower readily carbon utilization in majority soil samples especially after incubation in warmer climatic condition. This result explained that soil bacteria utilized readily carbon in lower level after incubation in warmer climatic condition. The lowest use of readily carbon in deciduous forest soils after the incubation might implies the bacteria use carbon intensively along the period, hence more prone to warming condition. Deciduous forest soils with typical more easily degradable organic matter than coniferous forest tend to be more prone under future climatic condition, suggested to temperature sensitivity on SOM decomposition. Decomposition of SOM is biological activity, differed below ground carbon allocation and nutrient availability under forest stands, that may result in different microbial composition and activity, resulting in different rate of SOM decomposition [49]. Higher SOM decomposition rate during warming condition in deciduous forest may affect more degradation of recalcitrant organic matter, hence increase the release of carbon stocks in the future climate.

As the resource was also fixed, finite and independent of temperature in the present study, higher temperatures more likely to result in higher rate of resource use from the beginning of the incubation. As a result, the active bacteria communities might change from communities which used easily degradable substrate into communities which used complex substrates. The change might indicated that bacteria communities in the prior incubation period were already inactive at the end of incubation. During the incubation, substrate change occurred, remained complex substrates had been used by bacteria. High rate of resource use through the incubation period in warmer condition seemed to give the result of lower resource availability after the incubation. In addition, bacteria communities isolated from temperate soils are usually found to have optimum growth temperature below 30°C and decreasing at higher temperatures [50,51], if the environment condition changes from initial optimum conditions, the composition of the bacteria community may shift. A rising temperature suggested to increase the metabolic activity, as a consequence, the adaptation of bacterial community after changing temperature may occur [26].
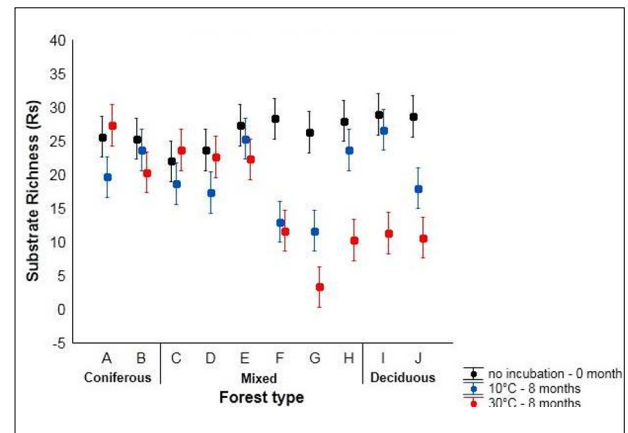

Fig 1. Two-way ANOVA for analysis carbon utilization calculated from metabolized substrates in Biolog® Eco Plate based on 96 hours incubation (n=90). The carbon utilization is calculated as substrate richness (Rs), the effect of incubation on Rs $F_{(18,60)} = 12.73$, $p < 0.0001$. The central points indicate the sample means, and error bars indicate 95% Tukey HSD intervals.

The change of metabolic profile in community level can be also determined by the analysis of relative utilization of different carbon sources on Biolog® Eco plates (Fig. 2). The analysis of substrate turnover of the six major substances groups showed that amines were degraded slowly after the incubation in all forest types, in contrast with polymers which were degraded faster after the incubation, especially at 30°C. The bacteria originating from coniferous and temperate mixed forest sites were found using the same carbon substrate, by the same pattern of amines, carbohydrates, carboxylic acids, polymers and miscellaneous. Whereas, bacteria communities originating from deciduous forest were found relatively different on carbon substrate utilization, showed very low after incubation at 30 °C, except in carbohydrates and polymers. The shift in communities into hardly degrading compounds due to limited easily degraded compounds occurred during incubation, and bacteria were adapted to become less active and use the carbon substrate in lower level. It is interesting that the carbon utilization calculated by substrate richness (R_s) and relative utilization of different carbon sources showed the same pattern that soil sample from deciduous forest differed from the other two types of forest. The result might implies that climatic

warming affect the shift of bacterial communities in deciduous forest soils become degrading more carbon complex, in the long term, may cause reduction of carbon stock in soils.

All substrates which were used differently by each incubation set also confirmed the change of bacterial communities on utilizing carbon source. Before the incubation, the active bacteria were the communities that degrade substrate readily such as amines, amino acids and carboxylic acids in high levels. The use of these groups of substrates changed into lower level after the incubation representing the altered bacterial communities which were captured.
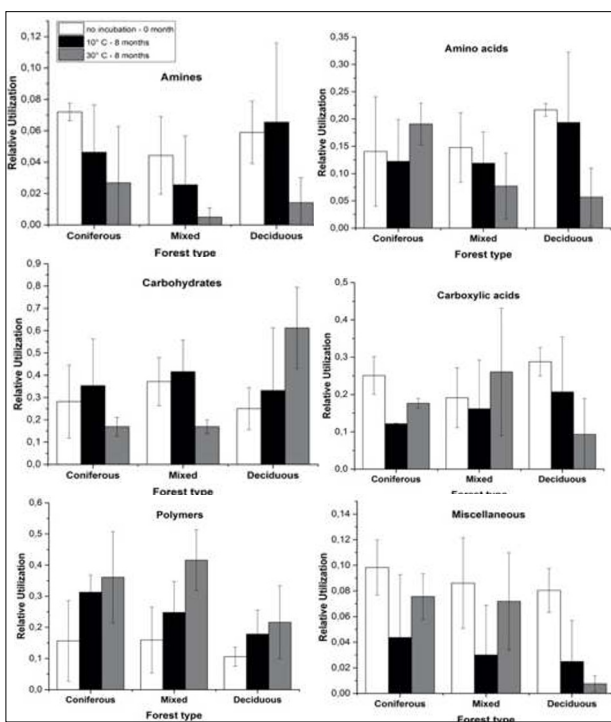

Fig 2. Relative utilization of different carbon sources (six major substance groups) in different forest types

The prior active bacterial communities were altered to the communities which degrade more complex substrates such as carbohydrates and polymers after the incubation. Especially the bacteria of deciduous forest that were characterized by the lowest use of amines, amino acids and carboxylic acids on Biolog® Eco plates. Moreover, carbohydrates and polymers substrate group showed the most decay rate by bacteria from deciduous forest soils after incubation at 30 ˚C, represented communities which used complex compounds more abundant, or switch into communities using more complex carbons as the easy-degradable carbons were run out. Among the forest types, bacterial communities originating from coniferous seemed to have more similarity on substrate preference with temperate mixed forest, meaning there were similarity of communities. While soil samples originating from

deciduous forest were found relatively different level, also very low carbon substrate were degraded especially after the incubation at 30 ˚C. Soil samples from coniferous forest exhibited higher substrate use after the incubation were correlated with their high availability of substrate.

Soil under different forest types performed different soil properties, related to litter production as dominating tree species affect soil characteristics through different quality of litter production [34]. Coniferous forest soils were observed as the most acidic among the forest types, while high in SOM content and C/N ratio. SOM is one of the main factors supporting the bacteria activity on degrading carbon substrate. In most soils, bacteria are carbon limited [35,36], higher organic carbon content in soil supports the development of larger and more active bacteria [2].
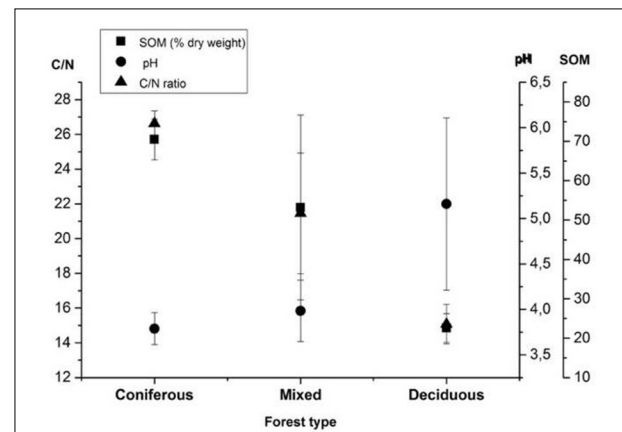

Fig 3. Soil organic matter (SOM), pH, and carbon/nitrogen (C/N ratio) in different types of forest. The study sites are defined into 3 types of forest, where coniferous forest consists of study site A and B; mixed temperate forest consists of study site C, D, E, F, G, H; deciduous forest consists of study site I and J.
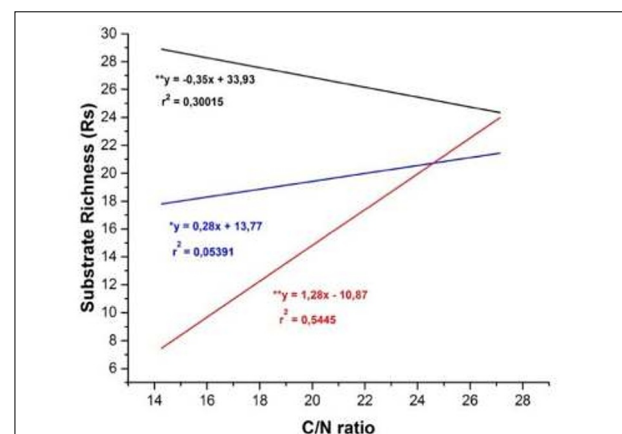

Fig 3. Relationship between substrate richness (Rs) and C/N ratio. Comparison of regression lines among bacterial community profiles for before the incubation (black line), after the incubation at 10° C (blue line), and 30° C (red line) along the C/N ratio ranges (n=30). Model of before and after incubation at 30° C were significant at p<0.05, the intercepts and slopes of regression lines for 3 different incubation sets differed statistically.

The relationship between soil bacteria carbon utilization and C/N ratio after incubation at 30 °C was significant ($p<0.05$, $r^2 = 0.545$). The C/N ratio is highly suggested as the main factor controlling the bacterial communities in the study sites. In most soils, bacteria are carbon limited [35,36], higher organic carbon content in soil supports the development of larger and more active bacterial communities [37]. Also, a study suggested the C/N ratio was one of main factors affecting bacterial communities structure [38]. The positive correlation in carbon utilization after incubation at 30 °C (Fig. 3) explained the enhanced temperature altered to raise the requisite of substrate. C/N ratio is related to availability of soil nutrient and as important factor contributing to the differences in composition of soil bacterial community in temperate ecosystems [39−42].
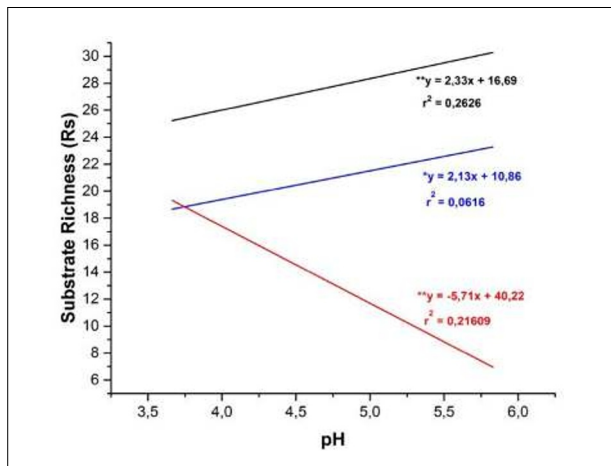

Fig 4. Relationship between substrate richness (Rs) and pH. Comparison of regression lines among bacterial community profiles for before the incubation (black line), after the incubation at 10° C (blue line), and 30° C (red line) along the pH ranges (n=30). Model with * was not significant, and ** was significant at $p<0.05$, the intercepts and slopes of regression lines for 3 different incubation sets differed statistically.

Soil pH is also known as one of the most important determinants of soil microorganisms condition [43]. Previous study showed forest type is one of the most influencing factors for soil [44]. Coniferous trees produce litter containing more lignin and leach more acids, thus worse decomposing than deciduous trees [45]. High pH is considered to be beneficial for the bacterial communities growth, thus supporting their activity. Previous studies have also suggested that bacterial growth is strongly affected by pH, increasing consistently with higher up to 6.5, and may start to decline toward more alkaline pHs [46]. The relationship between pH and soil bacteria carbon utilization was significant ($p<0.05$, $r^2 = 0.263$). Deciduous forest typically has high pH compared

to coniferous forest, owing to the large amount of leaf litter decomposition. Soil pH often been reported as significant factor affecting bacterial communities composition either directly [47] or indirectly through change of carbon and nutrient availability [48]. In this study, pH seems indirectly in relation with litters from different forest types. High pH in deciduous forest support high ability of bacteria to decompose more organic matter.

4. Conclusions

We observed bacteria carbon utilization related with soil physicochemical properties, and in relation with vegetation type through different climatic conditions. Litter production affected soil conditions, in which resulted in different soil properties such as pH, SOM, C/N ratio that act as limiting factors for soil bacteria activity on utilizing carbon sources. Deciduous forest performed distinct carbon utilization related to the soil physicochemical properties and vegetation types which were differed from two other forest types. Our results might become consideration for potential disturbance in organic matter cycling in the long term period.

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