Applied Soil Ecology 120 (2017) 30–34

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

Cellulolytic microorganisms control the availability of nitrogen in microcosm of shredded pruning litter treated highly acidic tea-growing soils of Assam in Northeast India

Prabhat Pramanik*, Shamina Safique, Ashrafa Zahan, Manabjyoti Phukan, Swayambhu Ghosh

Tocklai Tea Research Institute, Tea Research Association, Jorhat 785008, Assam, India

ARTICLE INFO

Keywords:
Shredded prunings
Cellulolytic microorganisms
Nitrate-N
Soil

ABSTRACT

Pruning (cutting the tea bushes from top) generates huge amount of organic residues in tea gardens and total nitrogen (N) content in those pruning litters is more than 10.0 mg g^{-1}. However, the hard nature of those pruning litters limits its application as an organic amendment in soil. In this study, soil was incubated with shredded pruning litters to evaluate their effect on N availability in soil. The study indicated that pruning incorporation at 10 t ha^{-1} increased ($P \leq 0.05$) nitrate-N content in soil and the values nitrate-N were proportional to cellulase activity and the population of culturable cellulolytic microorganisms in soil. Cellulolytic microorganisms possibly play a key role to degrade shredded prunings, which in turn enhanced nitrate-N content in soil by hastening the mineralization of organic compounds of prunings.

1. Introduction

Botanically, tea (*Camellia sinensis*) is a shrub (Martin, 2007); however, it is cultivated as a bonsai for its young shoots under tropical or sub-tropical humid climatic regions. Tea plants (bushes) are periodically cut from the top (pruned) to rejuvenate the bush and to maintain an appropriate height for convenience of plucking the shoots (Baruah, 1989). Pruning techniques (light pruning, medium pruning and heavy pruning) may vary depending on the depth of cutting from the top and those in turn determine the composition (stem and leaf proportion) of pruning litters. Pruning practice (19,845–24,570 kg ha^{-1} during light pruning, 14,175–16,200 kg ha^{-1} for medium pruning and 41,715–60,210 kg ha^{-1} for rejuvenation pruning) generate huge amount of waste residue (pruning litters) in tea garden (Singha, 2016). Considering the huge area of tea gardens (average area of tea gardens in Northeast India is more than 200 ha), several tons of pruning litters (both leaves and stems) are generated in each tea garden. Though pruning litters have high nitrogen (N) content (> 10.0 mg g^{-1}), especially the hard stems of prunings take more than one year to degrade in soil under field condition. Such slow decomposition of hard stems (50–70% of prunings, weight basis) limits its utilization as an effective organic amendment in soils (Mulky and Sharma, 1993). Recent findings by Safique et al. (2015) suggested that shredding of pruning litters (hereafter referred to as 'prunings'), especially of the hard stems, positively affected its decomposition in soil. However, quantitative studies on nitrogen mineralization in soil amended with prunings and on the potential of prunings to enhance nitrogen (N) availability in tea-growing soils have not yet been conducted. The pruning litters are left in the field for their decomposition; though stems of pruning litters can be recovered even after one year. The hypothesis of this study was that bound organic-N of prunings will be mineralized in soil faster enough (within few months time duration) so that N released in the plant-available form could potentially be utilized by plants in soil. Verma and Venktesan (2001) determined that pruning generates on an average 20 t litters in each hectare oftea gardens. In this study, shredded prunings were applied in soil at three different doses to quantify changes in different plant-available forms of N and the values were compared with related microbial and biochemical properties in soil. The objective of this experiment was to evaluate the potential of shredded prunings to supply N in available form after application in soil.

2. Materials and methods

2.1. Materials used and experiment setup

After every pruning cycle (3–4 years), tea bushes in each tea garden are pruned during winter season and that generates huge amount of pruning litter in tea gardens. In this study, prunings (both leaves and stems) were collected from the Borbetta Experimental Tea garden of

* Corresponding author at: Soils Department, Tocklai Tea Research Institute, Tea Research Association, Jorhat 785008, Assam, India.
E-mail address: prabhat2003@gmail.com (P. Pramanik).

http://dx.doi.org/10.1016/j.apsoil.2017.07.026
Received 18 October 2016; Received in revised form 14 July 2017; Accepted 17 July 2017
Available online 04 August 2017
0929-1393/

Table 1
Some chemical properties of hard prunings.

Parameters	Quantification
Total organic carbon	549.1 ± 26.7 mg g^{-1}
Total nitrogen	20.6 ± 3.7 mg g^{-1}
C/N ratio	26.65
Total phosphorus	5.3 ± 0.8 mg g^{-1}
Total potassium	14.8 ± 1.5 mg g^{-1}

Tocklai Tea Research Institute (26° 41′ 44.76″N and 94° 43′ 40.93″E), Jorhat, Assam, India and transported to the laboratory for further processing. The prunings were dried in oven at 60 °C for three days, mechanically shredded (≤3 cm), and applied to tea-growing soil at 5 t ha^{-1} (T_1: 2.50 g kg^{-1} soil), 10 t ha^{-1} (T_2: 5.0 g kg^{-1} soil) and 20 t ha^{-1} (T_3: 10.0 g kg^{-1} soil); the treatments were performed in laboratory under controlled conditions. Some chemical properties of the applied prunings were presented in Table 1. Soil without prunings was also considered as a control (T_0). All the treatments were run in triplicates. A virgin soil was collected from Borbetta Experimental Tea Garden of Tocklai Tea Research Institute. The soil was ground, homogenized and sieved through 2 m mesh before using for this incubation experiment. Some basic information of the soil used for this study was presented in Table 1. For each set, 2.0 kg soil was kept in earthen pots of 5.0-l capacity and a separate set of soil was incubated for each treatment and interval during this study. Therefore, total 60 pots (4 treatments x 5 intervals x 3 replications) were incubated at the beginning of this microcosm study. Moisture contents in each treatment were maintained near field capacity by periodical sprinkling of water. Soil samples were collected at 30 days interval for a total duration of 150 days. Fresh and air-dried soil was used for microbiological (microbial activity, populations of culturable nitrifying bacteria and cellulolytic microorganisms; cellulase activity) and chemical (available N and nitrate N (NO_3-N) and dissolved organic carbon (DOC)) analyses, respectively. Other chemical parameters like pH, total organic carbon (C), available phosphorus (P) and exchangeable potassium (K) were measured at the end of this study.

2.2. Chemical analyses of soil samples

Soil organic carbon (C) was measured by following the standard dichromate oxidation method of Nelson and Sommers (1982). To determine dissolved organic C (DOC) content, fresh soil samples were homogenized with deionized water (soil: water = 1: 5, w/v basis) by shaking at 120 rpm for 1 h (Lu et al., 2011) and the supernatant was used for organic C determination. Available phosphorous (P) content of the samples was determined by molybdenum blue method after the extraction with Bray's No. 1 reagent (Bray and Kurtz, 1945). Exchangeable potassium (K) content was determined by flame photometer from the neutral normal ammonium acetate extract of soil samples (Thomas, 1982).

Available nitrogen (N), nitrate-N and ammonium-N contents in soil were determined following the methods of Jackson (1973). Soil was extracted with 2 M KCl solution (soil: extractant = 1: 10, w/v basis) and the filtrate was then vortexed after adding 1 ml of 30% NaCl solution and 5 ml of 4: 1 sulphuric acid solution. The solution was then cooled down and 0.25 ml of colouring reagent (1 g brucine and 0.1 g sulphunic acid was dissolved in 100 ml of 3% HCl solution). The solution mixture was then warmed in water-bath at 100 °C for 20 min, cooled down and vortexed well to read the absorbance at 410 nm. The sample readings were then compared with that of standard curve to determine NO_3-N content in soil samples (Jackson, 1973).

2.3. Biochemical and microbiological analyses of soil samples

Five grams soil were placed in a 500 ml respiration flask and moistened with 1 ml sterile distilled water. The flask was incubated at 30 ± 1 °C for 5 h and a blank was also incubated without soil. The evolved CO_2 was absorbed in a 0.05 N NaOH solution and basal microbial respiration was determined by back-titrating the alkali solution against 0.05N H_2SO_4 solution. For determining substrate-induced microbial respiration (SIR), 1 ml of 1% glucose solution was added to soil before incubation (Anderson, 1982).

Populations of culturable nitrifying and cellulolytic microorganisms were determined by microbial plate counts following the method of Trevors and Cook (1992) and in this study, colony-forming unit (CFU) counts of above-mentioned microorganisms were referred as their abundance in soil. The population of culturable nitrifying bacteria was determined by growing them on selective Ashby's Mannitol Agar media (Subbarao, 1988). For screening nitrifying bacteria, fresh soil was suspended in 100 ml sterile distilled water and 500 µl of 10^4 times diluted soil suspension was spread on Ashby's Mannitol Agar media. The pH of the media was adjusted to 7.4 ± 0.2 before autoclaving and the plates were incubated at 28 °C and growth of bacterial colonies was observed after 7 days of incubation.

Soil (1 g) was stirred (80 rpm for 2 min) with 100 ml sterile distilled water in a conical flask and allowed to settle for 15 min. The suspension was serially diluted 10^5 times for determining the population of cellulolytic microorganisms. 500 µl of those solutions were spread on asparagine medium before pouring. Asparagine medium was prepared using following composition (Eggins and Pugh, 1962) (g l^{-1}): $(NH_4)_2SO_4$-0.5, L-asparagine - 0.5, KH_2SO_4-1.0, KCl - 0.5, $MgSO_4$-0.2, $CaCl_2$-0.1, yeast extract - 0.5, carboxymethyl cellulose (CMC) - 10.0 and agar - 15.0. The plates were incubated at 27 ± 1 °C for 48 h. To estimate cellulolytic microbial count, the CMC plates were stained with 0.1% aqueous Congo-Red solution followed by washing with 1 M NaCl solution. The clear zone indicates the presence of cellulolytic microorganisms.

Cellulase activity was measured by dinitrosalicylic acid (DNS) following the method of Ghosh (1987). 0.5 g soil was incubated with citrate buffer and 0.5 ml of 1% carboxymethyl cellulose (CMC) solution at 55 °C on water-bath for 25 min. After incubation, 0.5 ml DNS solution was added and the solution was again warmed for 5 min 1.0 ml Rochelle salt solution was added and solution was cooled at room temperature. Then the solution was diluted up to 10 ml and absorbance was measured at 540 nm. The concentration of reducing sugar was measured against glucose as standard and the cellulase activity was estimated as µg glucose released due to incubation of unit amount of soil for unit time interval.

2.4. Statistical analysis

The data were analyzed using standard statistical methods following the procedures of Gomez and Gomez (1984). Differences between treatments were determined by analysis of variance (ANOVA) and least significant differences (LSD) test using SPSS 14.0 statistical software. All statistical considerations were based on $P < 0.05$ significant level.

3. Results

3.1. Changes in available forms of N in soil

Concentrations of available N and nitrate-N were gradually increased in all the treatments throughout the study period (Fig. 1). Available N and nitrate-N contents in soil proportionately increased with increasing amount of applied prunings. At the end of five months experiment (T_3), concentrations of available N (209.4 mg kg^{-1}) and nitrate-N (102.5 mg kg^{-1}) in soil were 67% and 70% higher than those of control (T_0) soil, respectively.