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Nitrogen amendment of green waste impacts microbial community, enzyme secretion and potential for lignocellulose decomposition

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ABSTRACT

Microorganisms involved in biomass deconstruction are an important resource for organic waste recycling and enzymes for lignocellulose bioconversion. The goals of this study were to examine the impact of nitrogen amendment on microbial community restructuring, secretion of xylanases and endoglucanases, and potential for biomass deconstruction. Communities were cultivated aerobically at 55 °C on green waste (GW) amended with varying levels of NH_4Cl. Bacterial and fungal communities were determined using 16S rRNA and ITS region gene sequencing and PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was applied to predict relative abundance of genes involved in lignocellulose hydrolysis. Nitrogen amendment significantly increased secretion of xylanases and endoglucanases, and microbial activity; enzyme activities and cumulative respiration were greatest when nitrogen level in GW was between 4.13–4.56 wt% (g/g), but decreased with higher nitrogen levels. The microbial community shifted to one with increasing potential to decompose complex polymers as nitrogen increased with peak potential occurring between 3.79–4.45 wt% (g/g) nitrogen amendment. The results will aid in informing the management of nitrogen level to foster microbial communities capable of secreting enzymes that hydrolyze recalcitrant polymers in lignocellulose and yield rapid decomposition of green waste.

1. Introduction

Each year, 167 million metric tons of municipal solid wastes are sent to landfills in the U.S and about 44.9% of these wastes are organic [1]. While infrastructure has been developed in states such as California to recycle these materials using composting and anaerobic digestion, utilizing organic wastes for biofuels could also divert biomass from landfills, although the costs associated with waste collection and separation must be considered [2]. Among the currently collected and transported organic wastes, green wastes (GW), which include mixtures of leaves, grass, prunings and trimmings, and branches and stumps, are potential sources for compost and liquid fuel production in or near urban areas and they could be utilized locally to avoid transportation costs [3].

Efficient conversion of lignocellulosic organic wastes such as GW to compost and liquid fuel requires enzymes that function at extreme conditions, such as high temperature, high-solid loading, and low moisture [4–6]. Nitrogen is one of the essential nutrients for the growth of microorganisms and a critical element in enzymes, however nitrogen concentration is typically low in many terrestrial ecosystems and lignocellulosic waste streams [7]. Carbon to nitrogen ratio (C/N) is one of the important factors affecting composting processes; C/N ratios of 25–30 are usually considered ideal and blending of feedstocks is often required to achieve optimum ratios [8–10]. Nitrogen amendment has also been shown to affect activities of enzymes in soil ecosystems; cellulase activity of degrading dogwood, maple, and oak litter was stimulated by nitrogen addition while lignin degrading activity was significantly reduced in high lignin containing oak litter [11]. Although studies have shown the availability of nitrogen can alter structure, function,

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and enzyme activity of soil microbial communities [7,11,12], few studies have investigated the impact of nitrogen amendment on thermophilic microbial communities found in high solids biomass conversion systems and secretion of enzymes required for the hydrolysis of recalcitrant polysaccharides.

The goals of this study were to elucidate the biological mechanisms of enhanced GW decomposition with nitrogen amendment through examination of xylanase and endoglucanase secretion, microbial community restructuring and potential for GW deconstruction. Experiments were completed in a high-solids and aerobic environment at 55 °C with varying levels of nitrogen amendment. Compositions of both bacterial and fungal communities were examined and PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was applied to predict bacterial genes involved in lignocellulose degradation. The results will aid in informing the management of nitrogen to foster microbial communities capable of secreting enzymes that hydrolyze recalcitrant polymers in lignocellulose and yield rapid decomposition of green waste.

2. Materials and methods

2.1. Materials

Finished green waste compost was used as inoculum and obtained from a commercial facility that composts agricultural residues including tree and vine prunings (Northern Recycling, Zamora, CA). Compost was solar-dried and stored at 4 °C until applied as inocula.

Green waste was prepared based on composition data reported in the California 2008 Statewide Waste Characterization Study (CSWCS) [13]. In the CSWCS, California was divided into five regions including the Bay Area, Coastal, Mountain, Southern, and Central Valley. To simulate the municipal green waste in urban areas, only the composition data from the Bay Area and Southern Area were applied. The composition data from both regions were normalized to 100% and then averaged, resulting in the GW which had 53.4% (wet basis) leaves and grass, 38.2% (wet basis) prunings and trimmings, and 8.4% (wet basis) branches and stumps (Table S.1). The category of leaves and grass was further separated by assuming that the mass fractions of leaves and grass were 46% and 54% respectively, and therefore the mass fractions of leaves and grass in GW became 24.6 and 28.8% (wet basis), respectively (Table S.1).

2.2. High solids incubations

High-solids incubations were conducted as described previously using bioreactors with a 0.2 L working volume loaded with 6 g dry weight of GW (5.4 g) and inocula mixture (0.6 g) [14]. To prepare mixtures NH_4Cl was added to M9 minimal media to the target nitrogen concentrations of 1, 2, 3, 4, and 5 (g NH_4Cl/kg M9) as N-M9 solutions. The pH of each N-M9 solution was adjusted with HCl to match the pH of the standard N-M9 solution (pH 5.5), which was 1 g NH_4Cl/kg M9. Prior to the experiment, GW samples were wetted with the assigned N-M9 solutions to a moisture content of 400 wt% dry basis (g N-M9 solution (g dry solid)$^{-1}$) and equilibrated at 4 °C overnight.

An enriched community (T1) was established prior to the experiment to use as inoculum. Previous studies showed that the largest change in the microbial community composition occurred in the first enrichment [15] so only one enrichment was completed for this study. GW was wetted with 1 g NH_4Cl/kg M9 and inoculated with 10 wt% (g dry compost (g dry solid)$^{-1}$) compost for one week. After the incubation, each N-M9 treated GW sample was inoculated with 10 wt% (g dry enriched sample (g total dry weight)$^{-1}$) of the enriched community (T1) and transferred to a new bioreactor (T2). The T2 incubation ran for 11 days and the experiment was conducted with three replicates.

2.3. DNA extraction and 16S rRNA gene and ITS region sequencing

At the end of the each enrichment, 3 g wet weight enriched feedstock was collected from each reactor, frozen in liquid nitrogen, homogenized with an oscillating ball mill (MM400, Retsch Inc., Newtown, PA), and extracted using the MoBio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). The quantity of the purified products was measured with a Qubit® fluorometer (Life Technologies, Grand Island, New York) using the manufacturer's protocol. Three replicates were analyzed for each treatment. Sequencing was performed on purified DNA as previously described [16].

2.4. Enzyme extraction from solid samples

3 g wet weight of incubated GW was collected from each reactor at the end of the experiment. Samples were flash frozen with liquid nitrogen and stored at −20 °C for a week before enzyme extraction. Enzymes were extracted from incubated GW with a buffer containing 1 wt% sodium chloride, 0.1 wt% Tween 80 and 50 wt% ethylene glycol [6]. Endoglucanase (as carboxymethyl cellulose) and xylanase activities were measured as described previously [6]. All assays were completed in triplicate. Activities were reported as IU g dw^{-1} where one IU = μmol product min^{-1}.

2.5. Prediction of gene content

16S rRNA gene sequencing reads were processed using Quantitative Insights Into Microbial Ecology software (QIIME) [17]. Reads were filtered and trimmed following the default parameters (r = 3; p = 0.75 total read length; q = 3; n = 0; c = 0.005%) [18]. Closed-reference OTU picking was conducted within QIIME using the pick_closed_reference_otus.py command with default parameters. The Greengenes Database (Version 13.8) (pre-clustered at 97% similarity) was used as the reference sequence set [19]. The OTU table generated by QIIME was used as input for gene content prediction in PICRUSt software (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) [20]. To account for variation in the number of 16S rRNA genes between different taxa, OTU counts were normalized within PICRUSt based on predicted 16S rRNA gene copy numbers to better estimate the relative abundance of target genes [21]. Normalized OTU counts were used to predict the presence and abundance of genes belonging to protein families involved in cellulose, hemi-cellulose, lignin, and cello-oligosaccharide degradation.

2.6. Data analysis

Respiration data from high solids incubations were used to calculate CO_2 evolution rates (CER) and cumulative respiration (cCER) from CO_2 concentration and mass flow rate measurements of reactor effluents, as described previously [6]. Three replicate measurements were done for all nitrogen concentrations except for 3 g NH_4Cl/kg M9 which had only 2 replicates, due to a water clog inside the tubing connecting the reactor to the CO_2 sensor.

Sequences obtained through high-throughput 16S rRNA gene and ITS region sequencing of isolated DNA were quality trimmed, filtered, assembled and assigned to OTUs using methods described previously [16]. 16S rRNA gene and ITS region and read counts were used to conduct ecological and ordination analyses as described previously [22].