Enzymatic Activity and Microbial Biomass in Soil Amended with Biofuel Processing Byproducts

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Key Words: Distillers grains, thin stillage, glycerol, alkaline phosphatase, protease, dehydrogenase, microbial biomass.

Abstract

Plant essential nutrient and carbon contained in byproducts associated with biofuel production have increased their value as soil organic amendments. These byproducts include wet distillers grain, and thin stillage from ethanol production, and glycerol from biodiesel production. However, the potential of using these organic materials as soil amendment has not received enough attention yet. As a consequence, this study aimed to assess the impact of wet distillers grain, thin stillage and glycerol applied at three rates equivalent to 100, 200 or 400 kg N ha-1 in case of WDG and TS or 40, 400 or 4000 kg C ha-1 applied alone or combined with 300 kg N ha-1 as urea in case of glycerol on enzymes activity of alkaline phosphatase, protease and dehydrogenase, and microbial biomass C and N content. Urea and dehydrated alfalfa were also applied at three rates of N for comparison as conventional amendments and reference materials. Amended soil was incubated in controlled growth chambers for 10 days. Addition of urea, DA, WDG and GL+N significantly enhanced phosphatase activity especially at the low rate. Protease activity was significantly enhanced by all amendments addition especially glycerol. All amendments increased dehydrogenase activity, especially TS treatments. With the exception of TS, all amendments showed variable effect but significant on MBC and MBN content. The significant impact of BPB on measured microbial parameters is probably as a consequence of their effect on microbial growth and activity.

Introduction

Increased demand for sustainable and renewable source of energy, uncertainty of petroleum reserves and fluctuating oil prices have driven many countries to find alternative fuel sources. Therefore, ethanol was one of the most common alternatives whose production has expanded rapidly in recent years to meet world's energy growing needs. In 2007, biofuel industry generated approximately 49,587 million L worldwide, 24,602 million L in U.S. and 798.63 million L in Canada (RFA, 2007) and Canadian production of ethanol is expected to reach 1,777 million L by 2010 (USDA, 2007). This increase trend in biofuel production has resulted in large amount of ethanol and biodiesel byproducts being generated during biofuel manufacturing. As the ethanol production from cereal grain (mainly corn) involves the conversion of starch to ethanol through fermentation followed by distillation process, the byproducts of these processes are wet distillers grains; which is made of coarse grain particles, and thin stillage; which contains yeast cells, soluble nutrients and very small grain particles (Bonnardeaux, 2007). A bushel of

corn processed for ethanol production generates approximately 10.6 L of ethanol and more than 17 pounds of distillers grains; approximately 14.6 million metric tons of distillers grains were produced from U.S ethanol biorefineries in 2007 (RFA, 2007). The Biodiesel manufacturing also is accompanied by a primary byproduct of glycerol (also know as glycerin) produced via the transesterification of oils from plants (The Glycerol Challenge, 2007). Glycerol byproduct comprises 10% of biodiesel production; every tonne of biodiesel produced is associated with 100 kg of glycerol as a byproduct (The Glycerol Challenge, 2007). There are some studies carried out on glycerol such as its effectiveness on pig growth performance (Lammers et al., 2007; Groesbeck et al., 2008) or its traditional uses which include food additive, industrial chemical and pharmaceutical preparations. While these potential uses exist, surplus glycerol generated from rapid growth in biodiesel production is currently disposed of by incineration (Glycerol Challenge, 2007). However, research is underway to find alternative uses, including transformation into other value-added molecules. As it is a rich-carbon substrate, this would make it more attractive for being used as soil amendment; however, there is no information documented on its application to soil, and no attention has been given to this potential.

Due to their nutritional value of relatively high protein, phosphorus concentration and other minerals, distillers grains have commonly been used as animal feed (Ham et al., 1994; Bonnardeaux, 2006; Harris et al., 2008). However, continual rise in ethanol production may result in a surplus of distillers grains (Rausch and Belyea, 2006); therefore, alternative method of their utilization has to be found.

These byproducts are similar to other organic amendments such as animal manure, paper mill biosolids, sewage sludge, compost and crop residues; in terms of their content of essential plant nutrients. Many studies have investigated the effect of animal manure, sewage sludge, composts or paper mill biosolids on nutrient availability and soil fertility (Schoenau, 2006; Lupwayi et al., 2005;); soil enzyme activity (Mandal et al., 2007; Selivanovskaya and Latypova, 2006; Parham et al., 2002; Plaza et al, 2004; Garcı´a-Gil, 2000; Ferna´ndez et al., 2009); soil quality (Limon-Ortega et al., 2009).

As enzymes are considered to be important soil component, their activities in soil have potential to provide unique biological information of soils; therefore, they are attractive as one measure of soil health (Dick, 1997). The other microbial indices that have been suggested as soil health indicators are microbial biomass and microbial quotient; the amount of microbial biomass in soils reflects the total organic matter content (Sparling, 1997). The typical microbial biomass carbon comprises 1-5% (w/w) of total soil organic carbon, and microbial nitrogen comprises 1-6% of total soil organic N (Jenkinson and Ladd, 1981; Sparling, 1985; Wardle, 1992).

Nevertheless, several studies have investigated the effect of different types of organic amendments addition on microbial biomass and enzymatic activities in soil. However, information on application of biofuel byproducts on soil is scarce, as these organic materials are rich in carbon and other nutrients and their application to soil might stimulate microbial activity and soil organic matter turnover and therefore contribute to soil fertility and sustainability. This would open new avenues for methods of biofuel byproducts disposal that would be environmentally safe and economically sound. Therefore, the objective of current study was to investigate the effect of applying biofuel byproducts at different rates on microbial biomass C,

microbial biomass N, microbial quotient and activity of three selected enzymes in comparison to urea and alfalfa as conventional amendments.

Materials and methods

Experimental design

Soil and byproducts preparation

Soil selected for the incubation study was a fresh soil collected from the surface layer (0-15 cm) prior the incubation. It was a Brown Chernozem loam-textured soil. Wet distillers grain (solid) and thin stillage (liquid) from wheat-based ethanol production were provided by Pound-Maker ethanol production facility at Lanigan, Saskatchewan. Glycerol, a thick syrupy liquid from canola-based biodiesel production, was obtained from Milligan Biotech, Saskatchewan. Alfalfa used in this study for comparison was dried powder, dehydrated byproduct obtained from MCN Bioproducts Inc., Saskatchewan. All byproducts were sub-sampled for their chemical composition characterization and then stored at 4 °C until their use. Selected characteristics of the soil and byproducts used in the controlled environment chamber experiment are given in Table 1. The soil amendments were applied at three rates of urea, alfalfa powder, wet distillers grains, thin stillage and glycerol, which was applied with or without nitrogen. The three rates were equivalent to 100, 200 or 400 kg N ha-1 and this referred to as low, medium and high rate respectively in this study; however, the three rates of glycerol (low, medium and high) were 100, 1000 and 10000 kg glycerol ha-1 respectively equivalent to 40, 400, and 4000 kg C ha-1 respectively, given a C content of the glycerol of 40% C by weight. The rates of application were determined based on nitrogen content of amendments except the glycerol in which the rate was selected according to the carbon content since it does not contain nitrogen. Each rate of glycerol was applied alone or combined with 300 kg N ha-1 as urea to avoid N-limited decomposition (Recous et al., 1995).

Incubation set-up

Field-moist soil samples were weighed (650 g) and placed in 1-L pots. Three rates of urea (0.0864, 0.1728 and 0.3456 g/pot), dehydrated alfalfa (1.5773, 3.1564, and 6.3092 g/pot), wet distillers grain (4.36, 8.72 and 17.44 g/pot) thin stillage (8.512, 17.024 or 34.048 g/pot) and the glycerol treatments included three rates with equivalency to 100, 1000 or 10,000 kg glycerol ha^{-1} with or without 263.2 mg of urea (150 μg N g^{-1} or 300 kg N ha-1). A control that received no organic amendments was included. First, a 50 g of soil was mixed with the amendment and spread on the soil surface on the pot. Then 150 ml of deionized water, which is sufficient to bring soil moisture to field capacity level, was added and then 100 g of soil was placed on the top. In case of liquid or slurry amendments (glycerol, thin stillage), a 700 g of soil was weighed into each pot, and then the amount of amendment was mixed well with 150 ml of deionized water and then added to soil. Then, a 100 g of soil was placed on the top. Each treatment was replicated four times. All pots containing amended soil were placed on the bench in laboratory and remained in place for 6 h for stabilization prior their incubation. Pots containing amended soils were incubated for a period of 10 d in a growth chamber with electronically controlled environmental settings in which the chamber was set for 16 h at 25 °C (day) and 8 hrs at 18°C

(night). Moisture content levels in the pots were constantly maintained by measuring weight loss on a daily basis, and deionized water was added when needed At the end of incubation, soils were removed and sieved (< 2 mm) to determine enzymatic activity and microbial biomass C and N in each treatment.

Enzyme essays

Alkaline phosphatase activity was determined using p-nitrophenyl phosphatase substrate made in a buffer solution with pH = 11 as described by Alef et al. (1995). Briefly, 1 g of moist soil was treated with 0.25 ml of toluence, 4 ml of modified buffer (pH 11), 1 ml of p-nitrophenyle phosphate made in the same buffer, mixed and incubated for 1 h at 37 °C. After the incubation, 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH were added, and the content was mixed and filtered through a filter paper. The absorbance in the filtrate was then measured at 400 nm using spectrophotometer.

Dehydrogenase activity was determined by the reduction of 2,3,5-triphenylterazolium chloride (TTC) to triphenyle formazan (TPF) as described by Casida et al. (1964) and slightly modified by Serra-Wittling et al. (1995). In particular, 3 g of air-dried soil (< 2 mm) was incubated with 3 ml water and 3 ml TTC at 37 °C for 24 h in darkness. After incubation, 10 ml of methanol was added, the content was mixed and filtered through a glass fiber filter. Additional methanol was added until the reddish color disappeared from the filter. The filtrate was then diluted with methanol to a 100-mL volume. The intensity reddish color caused by the reduction of TTC to TPF was then measured using a spectrophotometer at 485 nm.

Protease activity was measured based on a method described by Alef and Nannipieri (1995). In brief, it was estimated by determination of the amino acids released from 1 g moist and sieved soil sample (< 2 mm) incubated with sodium caseinate (2%) for 2 h at 50 °C using Folin-Ciocalteu reagent. Centrifuged and filtered mixtures were read in a spectrophotometer at 700 nm.

Enzyme essays were conducted in duplicate with one control where the same procedure for enzyme essay was followed but the substrate was added to soil after incubation and subtracted from a sample value.

Microbial biomass analyses

The microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by fumigation extraction method as described by Voroney et al. (2008). Briefly, two 25 g portions of sieved soil (< 2 mm) that has been preincubated at 50% water holding capacity were weight out. One sample portion (25 g) was fumigated with ethanol-free CHCl3 for 24 h under vacuum and then extracted with 0.5 M K2SO4 (1:2 soil: extractant ratio). The other sample portion was extracted immediately. Total organic C and N from the fumigated and non-fumigated (control) soil extract were analyzed using CN analyzer (TOC-V_{CPH}-TN Shimadzu). The non-fumigated control values were subtracted from fumigated values, and MBC and MBN were calculated using K_{EC} factor of 0.25 for MBC (Wu et al., 1990; Joergensen, 1996) and K_{EC} factor of 0.18 for the MBN (Joergensen and Mueller 1996).

Statistical analysis

The experiment was set up in a completely randomized design. The treatments were arranged as a complete factorial. It consisted of 6 treatments with 3 levels plus a control. The effects of organic amendments, rate and their interaction on the activity of each selected enzyme, microbial biomass C, microbial biomass N and microbial quotient were carried out using the GLM model procedure in SAS software, version 9.2 (SAS Institute, Cary, NC). Means of the control and the 3 rates of each organic amendments application were separated by Fisher's protected LSD at $P < 0.05$ to test if they are significantly different. The effects were declared statistically significant at $P < 0.05$.

Results and Discussion

Enzyme Activity

Phosphatase activity was significantly influenced by amendments ($P < 0.0001$), rate ($P < 0.05$) and their interactions ($P < 0.0001$). The greatest value of phosphatase activity was obtained with dehydrated alfalfa applied at the low rate and decreased with increasing rate (Fig.1). The high rate of dehydrated alfalfa was not significantly different from the control. Urea treatments significantly affected phosphatase activity, comparing to the control, and similar results were obtained in case of WDG treatments (Fig.1). Neither thin stillage nor glycerol combined with nitrogen applied at three rates differed significantly from the control (Fig.1).

Dehydrogenase activity was significantly influenced by amendments ($P< 0.0001$), rate ($P< 0.0001$) and their interaction ($P< 0.01$). The highest value of Dehydrogenase activity was observed when thin stillage applied at a high or medium rate followed by dehydrated alfalfa or WDG, and urea with fluctuating values for each rate (Fig.2). The activity of dehydrogenase enzyme was higher when glycerol applied with N comparing to glycerol in absence of N (Fig.2).

Amendments ($P< 0.0001$), rate ($P = 0.001$) and their interaction ($P< 0.0001$) significantly affected protease activity in amended soil. Urea, dehydrated alfalfa and WDG amendments showed similar pattern in their effect on protease activity in which the tyrosine value increased with increasing rate (Fig.3). However, thin stillage impact was only significant when applied at a low rate (Fig.3). Glycerol addition stimulated protease activity whether in absence or presence of N (Fig.3), showing that the three rates of glycerol were significantly different from the control.

Microbial biomass C

The content of MBC was higher in soils received dehydrated alfalfa or WDG showing similar pattern of increase with increasing rate of application for both treatments (Fig.4). Urea application had also a significant impact on protease activity and similarly was observed with glycerol treatments in absence or presence of N. However, thin stillage showed no significant effect on protease activity when applied at any rate (Fig.4).

Microbial biomass N

The content of MBN in soils treated with dehydrated alfalfa or WDG at different rates increased with increasing rate and was higher in WDG-treated soil in particular at the high rate (Fig.5). The highest value of MBN in soil received urea was at the medium rate whereas low rate or high rate did not significantly differ from the control (Fig.5).

Conclusions

Generally, the significant response of microbial parameters measured in this study to monitor change in soil quality over 10 d incubation period indicated that biofuel processing byproducts application to soil stimulated microbial growth and activity, and thereby enhanced enzymatic activity and microbial biomass. The results of this study suggest that there is a high potential of using these organic materials as soil amendments, as alternative method of their use.

Acknowledgements: Financial support from Saskatchewan Agriculture Development Fund and ABIP Feed Opportunities for Biofuel Industry Program is greatly appreciated.

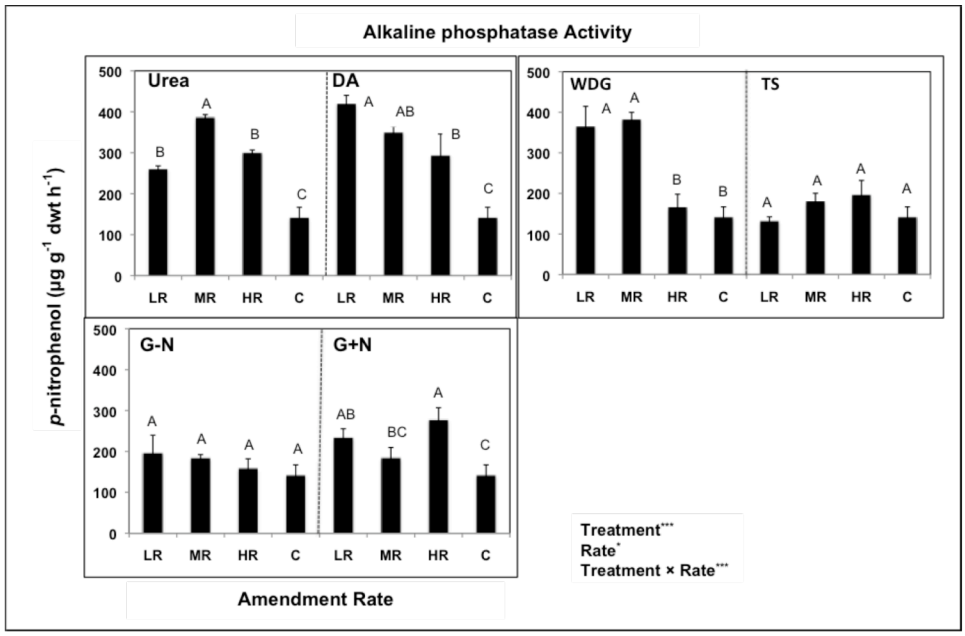

Fig. 1. Alkaline phosphatase enzyme activity in soil amended with 3 rates (low, LW; medium, MR; high, HR) of urea, dehydrated alfalfa (DA), glycerol without nitrogen (G-N), glycerol with nitrogen (G+N), wet distillers grain (WDG) and thin stillage (TS). Bars sharing the same letter within each treatment are not significantly different according to LSD test (P<0.05). Error bars represent standard error of 4 mean. *P<0.05; **P<0.01; ***P<0.001.

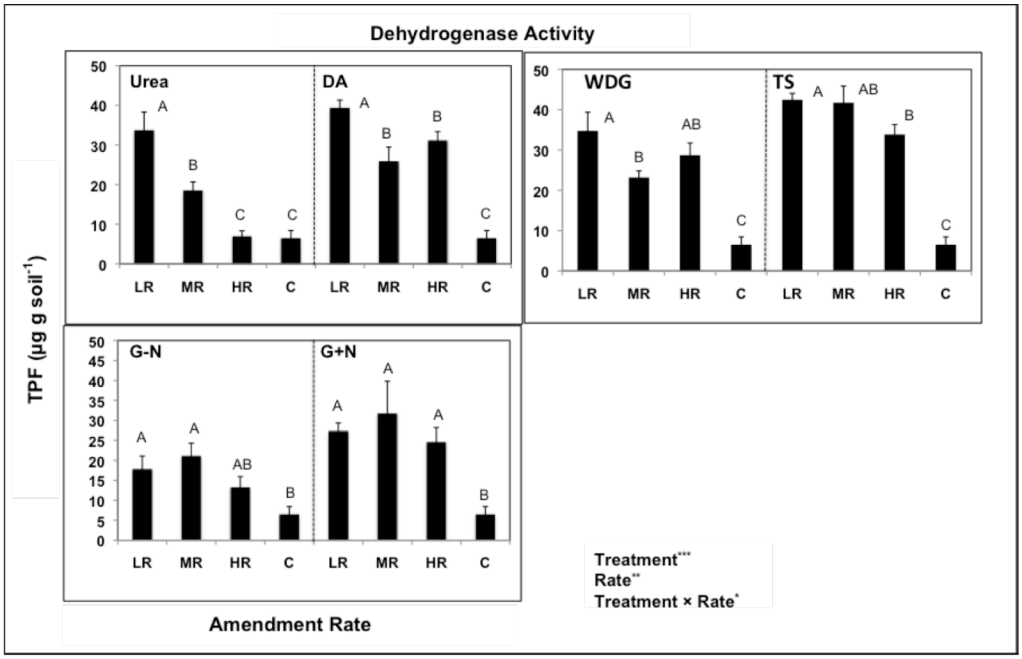

Fig. 2. Dehydrogenase enzyme activity in soil amended with 3 rates (low, LW; medium, MR; high, HR) of urea, dehydrated alfalfa (DA), glycerol without nitrogen (G-N), glycerol with nitrogen (G+N), wet distillers grain (WDG) and thin stillage (TS). Bars sharing the same letter within each treatment are not significantly different according to LSD test (P<0.05). Error bars represent standard error of 4 mean. *P<0.05; **P<0.01; ***P<0.001.

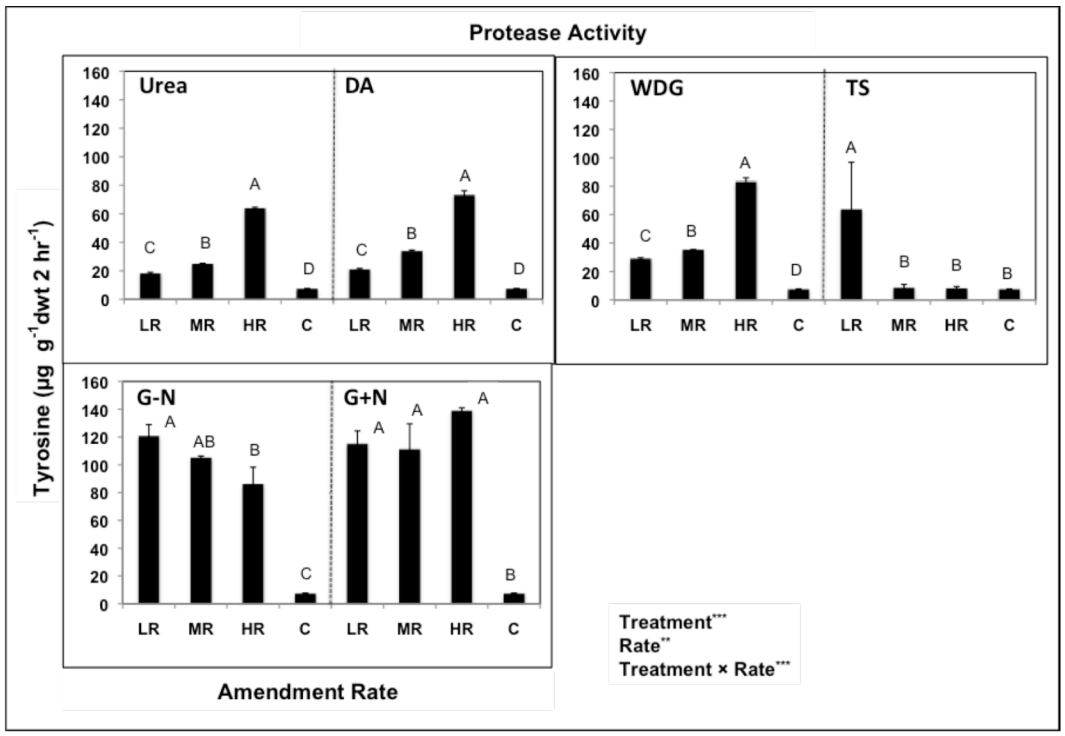

Fig. 3. Protease enzyme activity in soil amended with 3 rates (low, LW; medium, MR; high, HR) of urea, dehydrated alfalfa (DA), glycerol without nitrogen (G-N), glycerol with nitrogen (G+N), wet distillers grain (WDG) and thin stillage (TS). Bars sharing the same letter within each treatment are not significantly different according to LSD test (P<0.05). Error bars represent standard error of 4 mean. *P<0.05; **P<0.01; ***P<0.001.

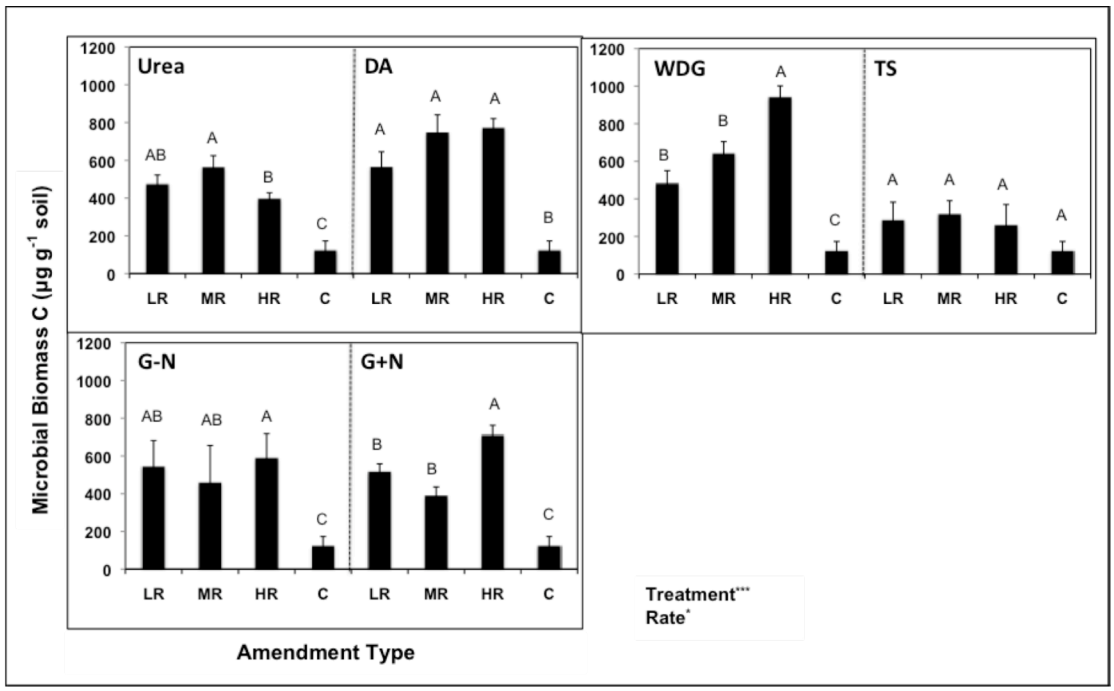

Fig. 4. Microbial biomass carbon (MBC) in soil amended with 3 rates (low, LW; medium, MR; high, HR) of urea, dehydrated alfalfa (DA), glycerol without nitrogen (G-N), glycerol with nitrogen (G+N), wet distillers grain (WDG) and thin stillage (TS). Bars sharing the same letter within each treatment are not significantly different according to LSD test (P<0.05). Error bars represent standard error of 4 mean. *P<0.05; **P<0.01; ***P<0.001.

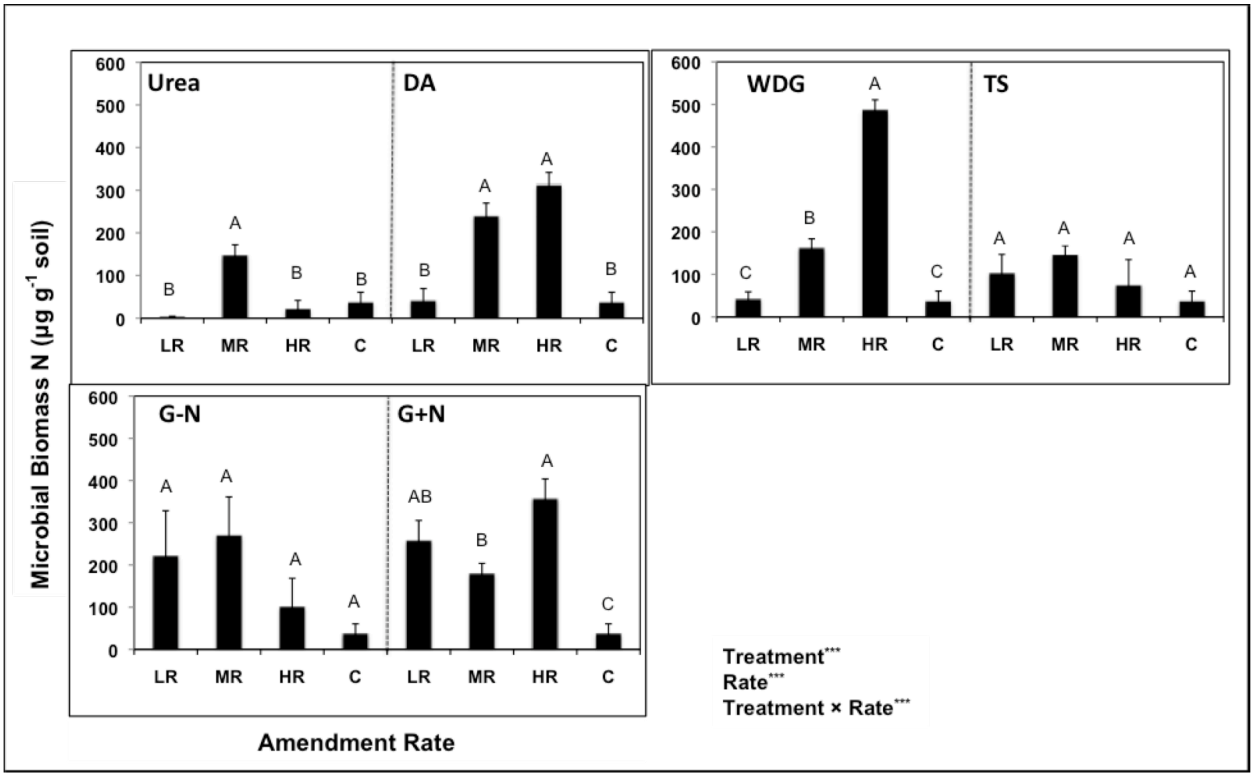

Fig. 5. Microbial biomass nitrogen (MBN) in soil amended with 3 rates (low, LW; medium, MR; high, HR) of urea, dehydrated alfalfa (DA), glycerol without nitrogen (G-N), glycerol with nitrogen (G+N), wet distillers grain (WDG) and thin stillage (TS). Bars sharing the same letter within each treatment are not significantly different according to LSD test (P<0.05). Error bars represent standard error of 4 mean. *P<0.05; **P<0.01; ***P<0.001.

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