INSTITUT FÜR
UMWELTWISSENSCHAFTEN
LANDAU

UNIVERSITÄT
KOBLENZ · LANDAU

Module: GEO 6 Soil Chemistry

Soil chemistry SS-2020

An abstract

on

FLOODING AND FERTILIZATION IMPACTS ON MICROBIAL CARBON AND NITROGEN BIOMASS OF SELECTED MEADOWS IN LANDAU

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Date of Submission: 29.11.2020

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1. Introduction: The source of the Queich River is in the Palatinate Forest of the South Rhineland-Palatinate and the location of the study was between Landau (49 ° 19′N, 8 ° 12′E) and Germersheim (49 ° 22′N, 8 ° 36′E) at the bottom of the Queich valley (Alt et. al., 2013; Buhk et al., 2018). Since 1990, traditional irrigation has been done twice or three times in a year in this region to make use of the fertilization effect from running water (Buhk et al., 2018). The effects of fertilization on the land in this region were well studied due to the intensity of agriculture. Also, the main goal of irrigation is to increase the biomass production in soil. (Müller et al., 2016) Irrigation can also change some chemical parameters of soil such as electrical conductivity, pH, organic nitrogen (N_{tot}) and carbon content (C_{tot}), nutrient availability (Mungai et.al., 2011). To investigate the impacts of irrigation and fertilization on selected soils, four samples were collected from four different field where three fields were treated (Flooded and fertilized, Non-flooded and fertilized, Flooded and non-fertilized) and one field was considered as "Control" (Non-flooded and non-fertilized). After collecting the soil samples, some physical and chemical parameters were determined. In this study, the main focus will be on microbial carbon (C_{mic}), nitrogen (N_{mic}) biomass and its explaining parameter. The total N_{mic} or C_{mic} of soil can be defined as the difference in the amount of nitrogen (N) or carbon (C) extracted from fumigated and non-fumigated soils with 0.5 M K_2SO_4 (Brookes et al., 1985). According to Li et al. (2018), different soil properties such as clay content, C_{tot} or N_{tot} are the main factors for having high microbial biomass in soil. Since organic matter acts as a source of energy for microorganisms, pH affects the size and shape of the microbial community, soil structure also affects the nutrient present in the soil and the water content that regulates microbial activity in the soil. (Li et al., 2018). The study also added that soil texture and organic material content have significant variance in N_{mic} and C_{mic} with significant P value ($P<0.05$). From the previous studies it had found that fertilization has negative effect on microbial biomass in soil (Söderström et al., 1983; Insam and Palojärvi, 1995) On the other hand, irrigation has a significant effect on increasing microbial biomass in the soil (Li et al., 2005). So, I hypothesized that fertilization has negative influence on microbial biomass (H_1) and irrigation has positive influence on microbial biomass(H_2). To evaluate the impacts of explanatory variables on microbial biomass, I asked the following question, (Q_1) which chemical and physical parameters of soil have most effect on the growth of microbial biomass in soil?

2. Materials and method: To collect soil samples from the selected sample sites, GPS machines were used to track the predetermined location of each sample site. Shovel, measuring tape, plastic buckets, PE bags were used to dig, pool and collect soil samples. After collecting soil samples, pH (in H_2O and $CaCl_2$), soil type, humus content was determined in the field following the "Soil Chemistry Manual (2020)". Soil samples were then prepared for further analysis in the laboratory. Sample preparation was accomplished by homogenizing and properly mixing of each soil sample for each treatment as well as the aggregates of soil samples were destroyed and large pieces of plant material were also removed. The different drying process of soil was done for different experiments such as field moist samples (stored in fridge at 5°C) were used to determine the effective cation exchange capacity, C_{mic} and N_{mic}. N_{mic} or C_{mic} was determined following the chloroform fumigation extraction method to destroy the cell walls of microorganisms and then instantaneous extraction was performed with 0.5 M K_2SO_4. C_{tot} and N_{tot} were determined by the dry combustion of soil sample at 105°C temperature in a CHNS Elementar Analyzer (VarioElementar). For statistical analysis, Shapiro-Wilk's normalization test was performed to test the normality of the data, non-normal data were box-cox transformed to get more accurate assumptions of normality. Two-way factorial analysis of variance (ANOVA) was done to see the overall effects of all treatments. Tukey's HSD test was conducted to detect the impacts of each treatment with the control. Multiple linear regression analysis was carried out to determine the impact of different physical and chemical parameters of soil on microbial biomass. A variance inflation factor (VIF) and co-linearity analysis were performed to check whether explanatory variable should be added or not in linear regression model. All statistical analysis was performed by R Studio^{R} (Version 1.2.5033).

3. Results: From the Shapiro-Wilk's test, it was clear that C_{mic} data were not normally distributed ($p = 0.02151$), but N_{mic} data were normally distributed (p-value = 0.7565). That´s why box-cox transformation had done for C_{mic} data. Figure 1 is a typical Q-Q plot of the C_{mic} and N_{mic} datasets where it shows a normal progression and thus follows a satisfactory linear trend. From the ANOVA test, it is clear that there are statistically significant differences between the treatments with significant p-value for C_{mic} ($p = 2.24\times10^{-8}$) and N_{mic} ($p = 1.96\times10^{-7}$).

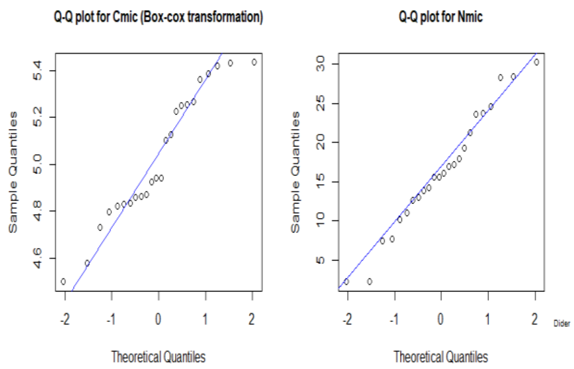

Figure 1: Q-Q plot for checking normality

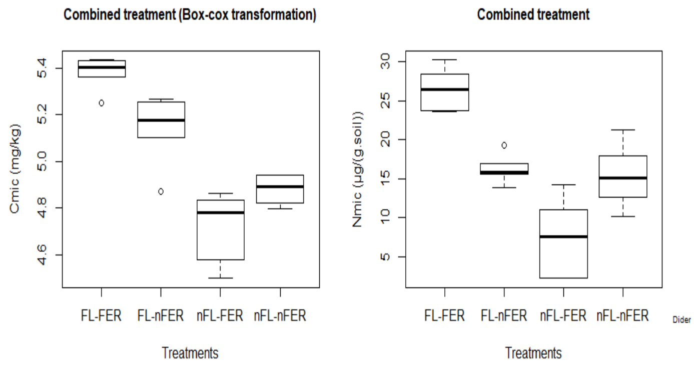

Figure 2: Microbial biomass for different treatments

Tukey's HSD test detect that flooding has a significant influence on the C_{mic} ($p = 0.0048$). Although there is no significant influence of fertilization on the C_{mic} (Figure 2), the combined treatment of flooding and fertilization have a more significant effect on the C_{mic} ($p = 2.1\times10^{-6}$). On the other hand, a significant effect of fertilization is found on the N_{mic} ($p = 0.005$). The combined treatment of flooding and fertilization have more significant effect ($p = 1.5\times10^{-4}$) on the N_{mic} (Figure 2) as well as no significant effect of flooding on the N_{mic}.

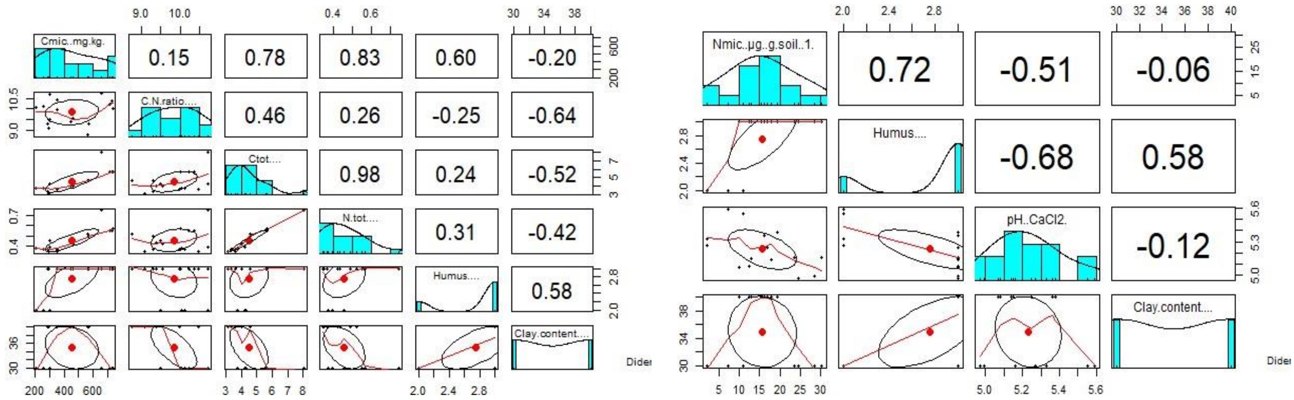

Figure 3: Correlation plot of explanatory variables for C_{mic} and N_{mic} response variables

Figure 3 shows that C_{mic} has high correlation with N_{tot}, C_{tot}, humus content with correlation coefficient 0.83, 0.78, 0.60 respectively and N_{mic} has only high correlation with humus content with correlation coefficient 0.72. VIF analysis had done with a set threshold of "10". VIF analysis of C_{mic} showed that C/N ratio (58.64), C_{tot} (1185), N_{tot} (1053), humus content (65.09) and clay content (21.04) have high significant VIF (VIF>10) than the other two pH and cation exchange capacity variables. That´s why we conducted our multiple linear regression model without pH and cation exchange capacity variables. Multiple regression model with selected variable resulted in a highly significant regression function with a significant p value (6.202e-05). From the linear model it observes that humus content has more significant influence on C_{mic} ($p = 0.0005$). C/N ratio, C_{tot}, N_{tot} are also significant with p-value of 0.0073, 0.0026, 0.0020 respectively and clay content is less significant than the other variables with significant p-value of 0.025. Linear regression model for N_{mic} response variables also resulted a highly significant regression function with a significant p-value (2.971e-08), when explanatory variables with non-significant p-value ($p>0.05$) were removed from the linear regression model. Regression model with selected variables detect that clay ($p = 9.83e-08$), humus content (1.07e-08) have more significant influence on the N_{mic} value than the pH ($p = 0.000116$).

4. Discussion: To visualize the actual effects of each treatment and to check whether hypothesis completely right or wrong, two boxplots had created with error bar as shown in Figure 2. From the boxplot it can be observed that there is a tendency to have lower C_{mic}, N_{mic} value on fertilized treatment and a tendency to have a higher C_{mic}, N_{mic} value in flooded treatment. With these results I can accept my hypothesis (H_1) which stated that fertilization has a negative influence on the C_{mic}, N_{mic} and also, I accept hypothesis (H_2) which stated that flooding has a positive effect on the C_{mic}, N_{mic}. According to Alt et al. (2018), microbial biomass activity was higher on the flooded lands than the non-flooded lands. The study also added that fertilized non-irrigated meadow has low microbial biomass due to tough compaction of the soil. A similar study was conducted in 1983 by Söderström, Bååth & Lundgren at south and central Sweden forests, the study found that the microbial biomass and microbial activity in the fertilized soils were decreased by 81 to 97% compared to controls. Another study that found a significant reduction in microbial biomass in the soil as a result of long-term application of fertilizer with urea or ammonium nitrate (Bååth et al., 1981). From Linear regression model and Figure 3, it can be said that humus content has more significant positive influence on C_{mic}, then C/N ratio, C_{tot}, N_{tot} also have significant positive influence on C_{mic}, clay content has less significant negative influence on C_{mic}. On the other hand, humus content has more positive significant influence on N_{mic}, clay content and pH have significant negative influence on N_{mic}. With these results the research question (Q_1) can be answered that humus content is the most explaining variables for microbial carbon biomass (C_{mic}), the second important explanatory variables are C_{tot}/N_{tot} ratio, total organic carbon content (C_{tot}), total organic nitrogen content (N_{tot}) and the clay content are the third explanatory variables. On the other hand, clay content and humus content are the most explaining variables for microbial nitrogen biomass (N_{mic}), the second important explanatory variable is pH. From Figure 3, it is also clear that C_{mic} has high correlation with N_{tot}, then with C_{tot} and humus content respectively. Although soil content has a negative correlation with C_{mic} and N_{mic}, there is a positive correlation between humus content (0.58) which is strongly correlated with C_{mic} and N_{mic}. A study was conducted by Li, Allen & Wollum in 2005 to investigate the effects of irrigation and fertilization on Soil microbial biomass, the study found that soil texture had 6.9 & 9.3% variance in C_{mic} and N_{mic} respectively with significant influence ($P<0.05$), organic material had 43.6 & 50.9% variance in C_{mic} and N_{mic} respectively with significant influence ($P<0.05$) as well as C:N ratio also had significant influence on C_{mic} and N_{mic} with significant influence ($P<0.05$). According to Holland & Coleman (1987), the application of moderate amount of nitrogen has increased the amount of microbial biomass of carbon and nitrogen in the soil. In our study, from the Dunn test (For non-normal data) or Tukey's HSD (For normal data) test of explanatory variables for different treatment, it was detected that the fertilization has a direct significant effect with significant p-value on the humus content ($p = 0.0093$), clay content ($p = 0.037$), pH ($p = 0.00377$) and also combined treatment of fertilizer and irrigation have a significant effect on the total nitrogen content ($p = 0.00218$), total carbon content ($p = 0.00218$). With these investigations, it can be said that the soil samples of our study were maybe fertilized with nitrogenous fertilizers mostly which have an indirect effect on the growth of C_{mic} or N_{mic} in the soil. Since, fertilization impacts on microbial biomass in soil depends on soil type, fertilizer type, nutrient application (Li et al., 2005).

5. Conclusion: In a nutshell, it is found that application of fertilizer has a negative influence on the C_{mic} and N_{mic} and flooding has a positive influence on the C_{mic} and N_{mic}. Humus content was a highly significant explaining parameter for the C_{mic}, followed by C/N ratio, C_{tot}, N_{tot} and also clay content. On the other hand, humus content was also a highly significant explaining parameter for the N_{mic}, followed by clay content and pH. In addition, it is observed that fertilization has a direct significant influence on humus content, flooding has no significant influence on humus content. But, combined treatment of fertilization and flooding have significant influence on N_{tot} and C_{tot}. So, it can be concluded that humus content, N_{tot} and C_{tot} are the most explaining parameter for microbial biomass which is influenced by fertilization as well as combined treatment of flooding and fertilization.

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