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Soil Microbial Community Response to Compost Addition to Nicosulfuron Contaminated Soil

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Abstract

The toxicity of nicosulfuron to none target organisms is its downside, which has generated concerns about the herbicide in spite of its high herbicidal activity. Practices that would facilitate accelerated degradation of this herbicide will certainly be complementary to its use. A completely randomized design laboratory incubation experiment was carried out to examine the potentials of organic and mineral fertilizers to stimulate microbial activities in soil under the influence of the nicosulfuron herbicide. Soil contaminated with the field rate of nicosulfuron was separately amended with compost and NPK mineral fertilizer, and the treated samples were incubated for 56 days at room temperature. Soil microbial activity and microbial biomass C were measured in dynamics for the period of incubation. Eco-physiological quotients were also computed at the end of incubation to determine responses of soil microbes at the community level to the treatments. Application of nicosulfuron alone was found to repress both microbial biomass and microbial activity.

Addition of fertilizer however caused these parameters to increase especially during the first 28 days after treatment application. The microbial metabolic quotient was raised by the soil amendments shortly after application with the exemption of NPK treated soil. However, only the soil samples in which compost was present lowered qCO_2 at the termination of the experiment. NIC-COMP and NIC-NPK respectively raised and lowered the soil carbon mineralization quotient (qM) measured at the end of incubation. The soil microbial community was also found to be positively affected by the addition of fertilizers as indicated by the Cmic: Corg ratio and the microbial biomass change rate quotient (qC). It was therefore concluded that though the nicosulfuron herbicide at the field recommended rate has potentials to negatively affect the soil microbial community, application of organic fertilizer may help the soil to regain its microbial competence through enhanced degradation engendered by biostimulation of native microorganisms.

Keywords: nicosulfuron, eco-physiological quotients, qCO_2, mineralization quotient, biostimulation

1. Introduction

The agricultural soil is the final destination of a large number of herbicides, whether they are soil or foliage applied. These herbicides, when applied to the field do not only control target weeds, but might also leave unwanted residues in the soil, which are ecologically harmful (Haney *et al*., 2000; Derksen *et al*., 2002; Riaz *et al*., 2007). Herbicides used for weed control in arable crops can exert very diverse influences on soil microorganisms, and as a result alter some biochemical processes in the soil. Previous studies have shown variability in the effects of sulfonylurea herbicides on the soil microbial activity. Bensulfuron-methyl, nicosulfuron and rimsulfuron treatments have been found to decrease significantly the abundance of bacteria in top soil (Saeki & Toyota, 2004; Djuric & Jarak, 2006). Metsulfuron-methyl and rimsulfuron have also been observed to cause significant changes in the content of microbial biomass and enzymatic activity in soil (Vischetti *et al*., 2000; Zabaloy *et a*l., 2008). None of these studies have however considered the responses of soil microorganisms to this class of herbicides at the community level employing eco-physiological indices.

The concentration of microbiological parameters relative to soil weight are often measured or combined as indices to quantify the contributions of microbial biomass, population, and activity to the functions of soils in connection to nutrient cycling (Nannipieri, 1994) and environmental buffering. Combining microbial parameters rather than their individual measurement will provide ‘internal control’, such as combining microbial activity and population measurements to provide more sensitive indications of soil pollution than either activity or population measurements alone (Brookes, 1995). As an example, the microbial metabolic quotient (qCO_2), has been proposed as an indicator of stress in soils (Anderson and Domsch 1993). Such approaches seem to be useful when attempting to understand the ecology of microorganisms in situ and their role in transformation processes in soil (Dilly and Munch, 1998).

Ecophysiological quotients are usually estimated by obtaining the ratio of physiological performances (respiration, growth/death, carbon uptake) to the total microbial biomass per

unit time (Moscatelli *et al*., 2005). These estimates have been advanced as better representations of the responses of soil microorganisms to alterations at the community level, because they provide comparison of microbial communities in quantitative terms (Anderson and Domsch, 1990).

Nutrient addition by way of fertilizer application to the soil has been found to have great influences on soil microbiological status, and may affect the composition of individual microbial community in the soil (Khonje *et al*., 1989; Sarathchandra *et al*., 1989 and Khamis *et al*., 1990). While research results have consistently revealed that adding organic manure of either animal or plant sources to soils increases soil respiration, microbial biomass and diversity (Goyal *et al*.,1999; Fontaine *et al*.,2003 and Jangid *et al*.,2008), opinions still remain divergent as to whether soil microbial community responds positively or otherwise to the application of mineral fertilizers (Dobbs, 1992 and Hyman *et al*., 1990). Application of chemical fertilizers stimulates the growth and multiplication of microorganisms, but increased dosage may inhibit the survival of microbes due to osmotic stress created by fertilizers (Bharathi *et al.* 2011).

Reconciling the documented harmful effects of the nicosulfuron herbicide with the aforementioned beneficial effects of fertilizers on the soil microbial community suggest that nutrient addition to the soil may provide cushioning to the detrimental consequences of the former on the soil microbial community.

The present study therefore seeks to examine the effects of the application of either organic or mineral fertilizer on certain soil microbiological indices in soil contaminated with nicosulfuron. In this study microbial metabolic quotient (qCO_2), carbon mineralization quotient (qM), microbial biomass change rate quotient (qC), and the ratio of microbial biomass C to organic C (Cmic:Corg) were determined to indicate the total energy expended per unit biomass, the fraction of mineralized C, the rate of C-loss/enrichment, and the potential of C to be mineralized respectively. Determination of these parameters is likely to provide more objective information on the status of whole community of organisms in the soil than those provided by individual parameter.

2. Materials and Methods

2.1 Chemicals

All the solvents and other chemicals used were analytical grades and obtained from Pascal Chemical Company Ltd. (Akure, Nigeria). The NPK fertilizer and Nicosulfuron herbicide used were obtained from local agro dealers in Akure, while the compost is a compost fertilizer formulation by the Ondo State Waste Management Board, Akure Nigeria (Sunshine Organic Fertilizer ®). The organic formulation contains 7% N and micro-nutrients.

2.2 Soil Sample

Soil was taken from the Crop Type Museum in the Experiment Station of the Department of Crop, Soil and Pest management, Federal University of Technology, Akure, Nigeria ($7^0$16 ℕ, $5^0$12 Ɛ). Soil was collected from a depth of 0-15 cm in an area with low level of organic

matter based on visual observation of the colour. The samples were brought to the laboratory in sealed polyethylene bags. The physico-chemical properties of the soil show 33.4% clay 47.6% silt 19% sand with 0.78% organic carbon, 1.1g/kg of soil total nitrogen, 8.5 mg/kg available phosphorus and 0.50 Cmol/kg available potassium. The pH of the soil was found to be 5.7 (1: 2 H_2O).

2.3 Soil Sample Preparation and Treatment Application

In the laboratory soil sample was passed through 2mm sieve and adjusted to 60% water holding capacity (WHC). They were then stabilized at room temperature in the dark for one week. 500 g of the stabilized soil samples was transferred into glass jars to which treatments were later applied. Treatments applied were Nicosulfuron alone at the field recommended rate of 40g a.i. ha^{-1} (Nic-NF), Nicosulfuron + Compost at 10 t ha^{-1} (Nic-Com), and Nicosulfuron + NPK 15:15:15 fertilizer at 200 kg ha^{-1} (Nic-NPK). The treatment also included a control soil that received no amendment (Control). The conversion of the field application rates of nicosulfuron and the fertilizers into mg/kg of soil was done assuming even distribution in 0-15cm of soil with a soil density of 1.5 g cm^{-3}. Prior to its application to the soil, calculated amount of nicosulfuron was dissolved in the water that was used to bring the soil to the 60% water holding capacity, while the compost and NPK fertilizers were calculated and added directly to the soil inside the jars. The control soil samples received equal volume of sterile distill water. The jars were then made airtight and incubated at room temperature for a period of 56 days. The moisture condition of the soils was maintained at 60% of maximum water holding capacity by the addition of sterile distill water at periodic intervals throughout the incubation period. To achieve this, the initial weights of the jars were determined and recorded at the beginning of incubation. These weights were confirmed from time to time, and any deviation, which indicated moisture loss, was corrected by adding water to arrive at the original weight. The experiment was set up as completely randomized design (CRD) and each treatment was replicated four times.

2.4 Determination of Physico-Chemical Properties of Soil

Soil texture, pH, Organic matter and soil nutrient status of the air dried soil sample were determined following standard methods (AOAC, 1990). The soil samples were analyzed for total N using Kjeldahl digestion and distillation method. Available phosphorus was by the Bray 1 method, exchangeable K, Ca and Mg were determined by extraction with 1M ammonium acetate at pH 7.0. K, Ca and Mg contents were determined with flame photometer. Soil pH (1:2 soil-water) was determined by pH meter, while organic matter (OM) was determined by dichromate oxidation method.

2.5 Measurement of Soil Basal Respiration (SBR)

Basal respiration (μg CO_2-C g^{-1} soil) was determined by the alkali sorption and titration method described by Anderson and Domsch (1990). Three day prior to sampling, a 10 mL solution of 0.5M NaOH was dispensed into a 50mL beaker and placed inside the glass jars containing the treated soil to trap CO_2 evolved from the soil. On the third day, 5mL of 1.0M $BaCl_2$ was added to the NaOH solutions from the jars to precipitate carbonate (as $BaCO_3$),

thus facilitating the determination of CO_2 evolution (as μg CO_2-C g^{-1} soil) from the treated soil. The evolved CO_2-C was then determined by titration. NaOH in solution was titrated against 0.5 M HCl using phenolphthalein indicator. Two blanks without soil were prepared to assess the amount of CO_2 trapped without respiratory activity.

2.6 Determination of Soil Microbial Biomass Carbon (C_{mic})

Soil microbial biomass C (Cmic) was determined by the fumigation and extraction method described by Vance *et al*. (1987). 10g of unfumigated soil was extracted with 50 mL of 0.5 M K_2SO_4 by shaking for 45 min with a rotary shaker at 180 rpm, and the suspension filtered using a Whatman No. 2 filter paper. A separate portion was fumigated by placing it in a 50-mL beaker inside a desiccator alongside with another beaker containing ethanol-free chloroform. The desiccator was covered and evacuated with a vacuum pump until the chloroform boiled vigorously for 5 min. The evacuation was repeated three times at intervals of 15 min, letting air pass back into the desiccator to facilitate the distribution of the chloroform throughout the soil. The desiccator was evacuated a fourth time until the chloroform boils vigorously for 2 min. 24 hours later, the $CHCl_3$ was removed by vacuum extraction and the fumigated sample was extracted as above. Organic carbon in the extract was determined by the wet combustion method of Walkley and Black. MBC was calculated by the differences between the fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor (Kc = 0.35) (Sparling *et al*., 1990).

2.7 Determination of Eco-physiological indices (qCO_2, qM and qD / qC, and Cmic:Corg)

qCO_2 (the community respiration per biomass unit or the metabolic quotient) and qM mineralization quotient), and Cmic:Corg were measured at the end of incubation. qCO_2 was determined as the ratio of cumulative CO_2-C (μg CO_2-C g^{-1} soil) to the soil microbial biomass carbon, while qM was determined as the ratio of CO_2-C (μg CO_2-C g^{-1} soil) to the soil organic carbon (mg g^{-1} soil). Estimation of Cmic:Corg was based on the ratio of soil microbial biomass carbon to the total organic carbon.

Determination of the microbial biomass change rate quotient (qC) which expresses the daily enrichment or loss of soil microbial C was calculated based on qD (death rate) as reported by Anderson and Domsch (1990). In this study, the biomass C (C_{mic}) measured at 7 days after treatment was used as the initial microbial biomass of the soil. The incubating soils were maintained at room temperature in the dark and C_{mic} was recorded at each time of sampling. The data were based on arithmetic means of four replicates soil samples. The C-loss quotient (unit C-loss $unit^{-1}$ C_{mic} residual h^{-1}) was calculated based on total microbial-C-loss after the number of days of incubation before sample collection using the following equation

$$qD = [(C_{mic})_{t1} - (C_{mic})_{t2}/(C_{mic})_{t1}] / t_2 - t_1.$$

In this study, the qC was preferred to the qD of Anderson and Domsch (1990) because both C-loss and C-enrichment were encountered in the effects of the treatment applied.

2.8 Data Analysis

Data collected from the experiment were subjected to an analysis of variance while treatment

means were compared using the Tukey test at 5% level of probability. Graphs were designed using Microsoft Excel (2016 version) and error bars were determined using standard error.

3. Results

3.1 Effects of Treatments on CO_2-C Production

Results summarizing the effects of the various treatments on soil respiration from 7 to 56 day after treatment (DAT) are presented in Table 1. Significant alterations in soil respiration were caused by the treatments only on the 7th, 14th and 21st day after treatment application. At 7 DAT, addition of fertilizer (irrespective of type) to nicosulfuron contaminated soil significantly increased soil respiration. The increase was however higher (64.2%) when compost was added. Application of the herbicide alone decreased CO_2-C production at this time, but the decrease was not significant compared to the non-amended soil. The same trend of influence also continued up to the 21st DAT. None of the treatments significantly ($P < 0.05$) altered soil respiration from 28 DAT up to the termination of the experiment. At the end of the experiment, all the treatments slightly lowered soil CO_2-C production compared to the control, though not significantly, and the treatment involving nicosulfuron alone recorded the lowest CO_2 evolution at this time.

Table 1. Treatment effects of nicosulfuron on soil respiration (CO_2- C mg kg^{-1} soil)

Treatments	Days after treatment application							
	7	14	21	28	35	42	49	56
NIC-NF	1.90^{c}	2.60^{b}	2.66^{b}	2.72^{a}	3.01^{a}	2.72^{a}	2.60^{a}	2.00^{b}
NIC-COMP	3.12^{a}	3.26^{a}	3.26^{a}	2.88^{a}	3.36^{a}	3.02^{a}	2.74^{a}	3.02^{a}
NIC-NPK	2.90^{ab}	3.04^{ab}	3.04^{ab}	2.76^{a}	3.20^{a}	2.90^{a}	2.74^{a}	2.90^{a}
CONTROL	2.08^{bc}	2.88^{b}	2.70^{b}	2.90^{a}	3.22^{a}	2.70^{a}	2.66^{a}	3.26^{a}

Means in each column bearing the same letters are not significant at the 5% level of probability by Tukey's test.

Significant variations were observed among the treatments up to the 35th day after treatment application regarding their influences on cumulative soil respiration (Table 2). The presence of fertilizer (either organic or inorganic) significantly increased cumulative soil respiration compared to the Nic-NF treatment up to the 28th day after treatment application. Addition of compost to the herbicide contaminated soil however produced the highest volume of cumulative CO_2-C at each time of sampling from the beginning to the end of incubation. The Nic-NF treatment also lowered cumulative microbial activity in most parts of the incubation period. None of the treatments applied significantly altered cumulative soil respiration from 42 DAT till the termination of the experiment.

Table 2. Treatment effects on the cumulative soil respiration

Treatments	Days after treatment application							
	7	14	21	28	35	42	49	56
NIC-NF	1.90^{c}	4.78^{b}	7.62^{b}	10.34^{b}	13.56^{b}	16.28^{a}	18.88^{a}	21.92^{a}
NIC-COMP	3.12^{a}	6.38^{a}	8.92^{a}	11.80^{a}	15.16^{a}	18.18^{a}	20.92^{a}	23.94^{a}
NIC-NPK	2.90^{ab}	5.94^{a}	8.94^{a}	11.70^{a}	14.90^{ab}	17.80^{a}	20.54^{a}	23.44^{a}
CONTROL	2.08^{bc}	4.56^{b}	7.86^{b}	10.76^{ab}	13.62^{b}	16.32^{a}	18.98^{a}	22.24^{a}

Means in each column bearing the same letters are not significant at the 5% level of probability by Tukey's test.

3.2 Effects of Treatments on Soil Microbial Biomass C (Cmic)

Addition of the herbicide alone repressed soil microbial biomass C from the 7th to 56th day after soil contamination. The presence of compost and NPK fertilizer on the other hand slightly increased soil microbial biomass (12.5 and 7.2 %, respectively) on the 7th day after application (Table 3). These trends were noticed to continue to the end of the experiment, and the rate of increase was positively related to time after treatment application. Addition of compost recorded the highest Cmic from the 7th day to the termination of the experiment compared with all the other treatments. Except in the soil sample to which compost was added, Cmic declined as incubation progressed from the beginning to the termination of the experiment.

Table 3. Treatment effects of Nicosulfuron on microbial biomass C (mg kg^{-1} soil)

Treatments	Days after treatment application			
	7	14	28	56
NIC -NF	38.0^{b}	34.2^{c}	22.8^{b}	19.0^{c}
NIC-COMP	68.4^{a}	87.4^{a}	72.2^{a}	72.2^{a}
NIC-NPK	65.2^{a}	64.6^{ab}	44.2^{b}	34.0^{b}
CONTROL	60.8^{a}	57.0^{bc}	34.6^{b}	22.8^{b}

Means in each column bearing the same letters are not significant at the 5% level of probability by Tukey's test.

3.3 Effects of Treatments on Eco-Physiological Quotients

All amendments raised microbial metabolic quotient (qCO_2) compare to the control at 7 days after application except when NPK was added to the nicosulfuron contaminated soil (Figure 1). The presence of NPK lowered qCO_2 but not significantly ($P < 0.05$) at this time. NIC-NF maintained increase of this quotient up to the end of incubation, whereas the presence of compost caused this parameter to reduce towards the end of the experiment. Addition of NPK on the other hand was the only treatment that significantly ($P < 0.05$) raised qCO_2 at the termination of the experiment. Variations among the treatments as touching their effects on the carbon mineralization quotient (qM) determined at the end of incubation were minimal (Figure 2). While the application of the herbicide alone did not alter qM compared to the non-amended soil, the NIC-COMP and NIC-NPK respectively raised and lowered the soil carbon mineralization quotient.

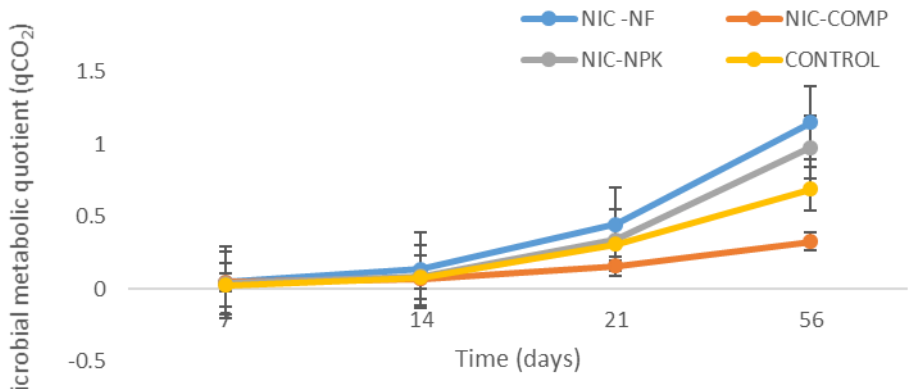

Figure 1. Treatment effects on microbial metabolic quotient (qCO_2)

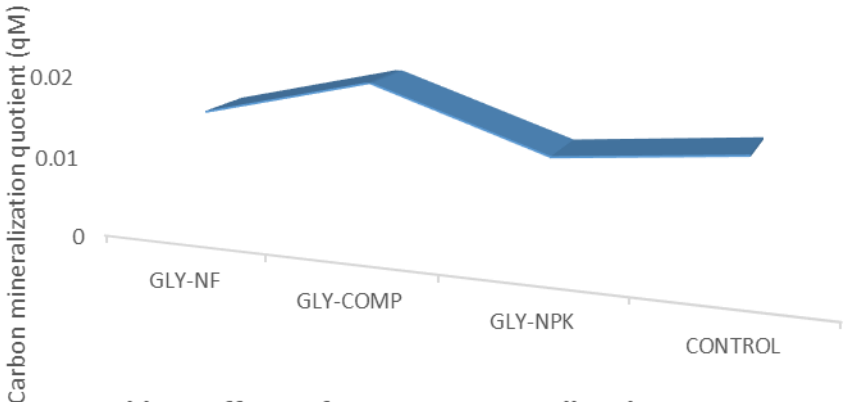

Table 2. Effects of treatments on soil carbon mineralization quotient (qM)

Results showing the effects of the treatments on the ratio of biomass C to organic C (Figure 5) indicates that this parameter increased in the order of NIC-COMP > NIC-NPK > Control > NIC-NF, and the observed differences were significant ($P < 0.05$). Response of the microbial carbon change rate quotient (qC) on the 14th, 28th and 56th day after incubation as presented in Figure 3 indicated that at the 14th day after treatment application, negative values were recorded for qC in both the treated and untreated jars with the exception of the compost aplication. The loss in carbon was however significantly ($P < 0.05$) lower in the NIC-NPK treatment compared with the control or application of nicosulfuron alone. Interestingly, between the 14th and the 28th day after incubation, death quotient (qD) scenario was observed across the treatments including the control, and percentage C loss relative to the non-amended soil was in the order 49 > 32 > 9 % for NIC-NF, NIC-COMP and NIC-NPK, respectively. The trend took another turn between the 28th and 56th day after incubation as C loss was almost at pal between the control and NIC-NPK treatment while C loss was greatly reduced (66.7 %) in the nicosulfuron treatment alone compared to the previous record. No loss or enrichment in C was recorded in the NIC-COMP treated soil sample within this period.

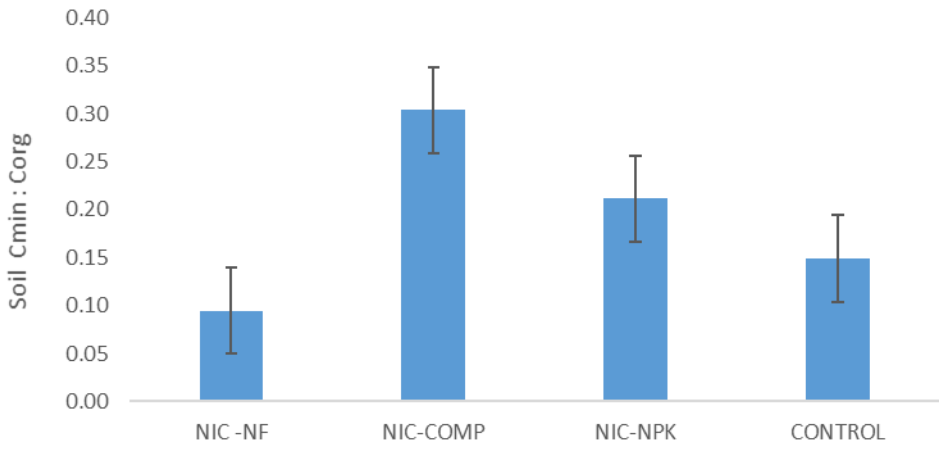

Figure 3. Effects of nicosulfuron-fertilizer treatments on the soil Cmin : Corg

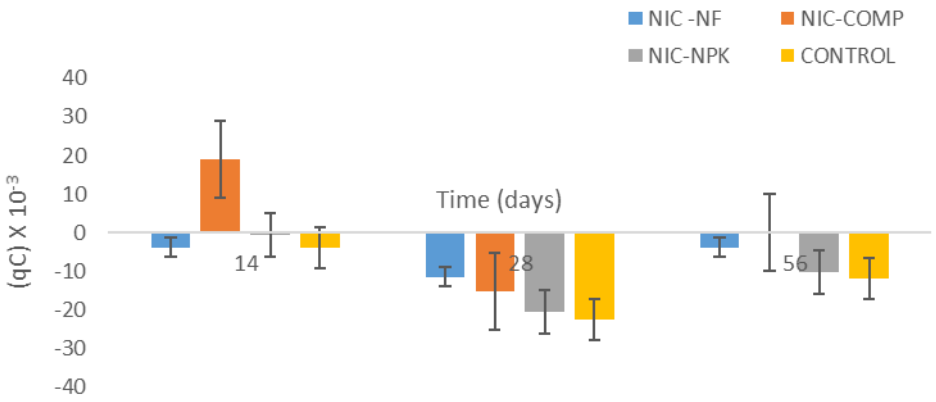

Figure 4. Effects of treatments on microbial biomass change rate quotient (qC)

Results of Soil pH and Total organic carbon (OC) determined at the end of incubation period (Figure 5) indicate none of the treatments significantly altered soil hydrogen ion concentration. Nicosulfuron applied alone or in combination with NPK fertilizer however slightly lowered this parameter while compost raised the pH of the soil. All the treatments increased total organic carbon at the termination of the experiment and the highest concentration (mg C kg^{-1} soil) of soil organic carbon was recorded in the soil treated with compost.

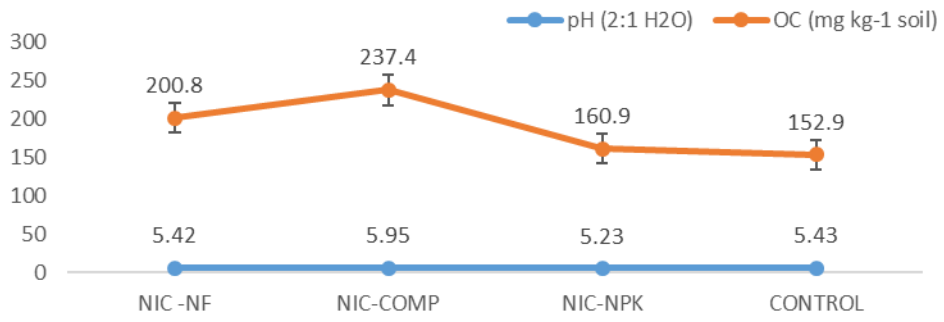

Figure 5. Effects of treatments on soil pH and Organic carbon

4. Discussion

The significant increase in soil respiration caused by the addition of fertilizer to nicosulfuron contaminated soil indicated that nutrient supplied by the fertilizers caused biostimulation of the native microorganisms in the soil. Addition of manures of both plant and animal origin to soil has been observed to increase microbial biomass-C and soil respiration (Tejeda *et al*., 2008), and these increases have been reported to be rapid upon addition of the nutrients (Goyal *et al*. 1999; Fontaine *et al*. 2003). The compost treatment

compared favourably with NPK in its biostimulation ability at 7 days after treatment application. This indicated that the Sunshine® organomineral formulation contained readily available nutrients, which is probably as a result of low C:N ratio. Soil microbial respiration, measured through CO_2 production is a direct indicator of microbial activity, and indirectly reflects the availability of organic material (Gomez *et al*., 2006; Tejada *et al*., 2006). The decrease in soil respiration engendered by the sole application of nicosulfuron can be associated with toxic effects of the herbicide on certain soil microorganisms, which probably did not adapt to the insecticide. This is in consonant with the assertion that nicosulfuron treatments have been found to decrease significantly the abundance of bacteria in top soil (Saeki & Toyota, 2004). These repressive effects of the herbicide on soil respiration however appeared to be transient because the soil recovered from this effect after the 21st day of incubation.

The drop in soil microbial biomass caused by contamination with nicosulfuron indicated that the native microbial community was dominated by those that could not readily metabolize the herbicide. The increase in biomass as a result of addition of fertilizer was attributed to the incorporation of easily degradable materials, which stimulated the authoctonous microbial activity (Blagodatsky *et al*., 2000; Tejada *et al*., 2006). The increase in soil microbial biomass caused by the compost treated soil above those treated with NPK fertilizer suggested that fertilizers of organic sources guarantee a more sustainable biostimulation of native microbial community. This was evident in the highest values of this parameter recorded against the compost treatment all through the incubation period. The sustained reduction in Cmic caused by nicosulfuron up to the end of incubation indicated that a period of fallow might be required before the introduction of another crop to follow in rotation when nicosulfuron is employed to control weeds in a short-season crop.

The increase in qCO_2 caused by nicosulfuron application alone and in combination with NPK at 7 days after treatment application validates the responses of the other microbial activity indicators (CO_2-C and C_{mic}) to these treatments. This also confirmed the depressing effects of the herbicide nicosulfuron on soil microbial biomass earlier observed (Saeki & Toyota, 2004). Increased qCO_2 indicated that the microbial community expended more energy per time. Anderson and Domsch (1990) has earlier pointed out that higher value of qCO_2 indicates more stressed conditions. The status of qCO_2 at the end of the experiment suggested that NPK imposed stress on the soil microbial community while the compost formulation created a more favourable environment for soil microbial activities. Differences among the various treatments were however not very pronounced regarding their effects on the proportion of soil organic carbon minerialized. This was indicated by their influences on the soil carbon mineralization quotient (qM).

The soil Cmic:Corg represent the potentials of soil microbes to mineralize and immibilize carbon. The increase in this parameter recorded in the presence of the compost formulation is an indication that the soil microbial community was able to immobilize more carbon for biomass production under the influence of compost. Toxicity of the herbicide to soil microorganisms should be responsible for the low carbon immobilization in soil to which nicosulfuron was applied alone, indicating that the soil microbial community was not able to

effectively metabolize the herbicide. This confirms the documented toxic influence of the sulfonylurea herbicides on soil microbial biomass (Vischetti *et al*., 2000). The apparent differences between sole nicosulfuron application and combination with fertilizer in its influence on soil carbon mineralization and C enrichment might be due to the presence of a microbial community, which presumably was capable of using the products from the metabolic decay of the fertilizers for biomass production. This probably conferred a cometabolic influence on the herbicide, to make it undergo degradation without being metabolized, a scenario that is likely to reduce the toxic effects of the herbicide on soil microorganisms. This is similar to the observation Adejoro (2016) when glyphosate was observed to serve as "body-guard" to facilitate cypermethrin degradation in a laboratory incubation study. Observation of carbon enrichment in the compost amended soil suggested that the compost formulation contained easily mineralizable carbon, which was quickly immobilized by soil microorganisms. C loss typifies microbial death quotient (qD), which was described by Anderson and Domsch (1990) as microbial carbon loss. This might have resulted from the exhaustion of carbon in the incubated soil samples.

Reduction of soil pH under the influence of NPK may be attributed to efficient assimilation of N by soil microorganisms leading to the production of acidic metabolites such as organic acids (He and Suzuki, 2004).

5. Conclusions

This work clearly demonstrated that the nicosulfuron herbicide at the field rate of 40g a.i ha^{-1} caused prolonged repression in soil microbial activity as measured by soil basal respiration as well as on soil microbial biomass carbon. A period of fallow is therefore prescribed to allow the soil microbial community recover from nicosulfuron toxicity before introducing another crop to follow in rotation after weed control with nicosulfuron. However, in the face of high pressure on available agricultural land, which has made land fallow practically impossible, application of organic fertilizer may help the soil to regain its microbial competence through enhanced degradation engendered by biostimulation of native microorganisms.

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