Agricultura 16: No 1-2: 35-42(2019)
https://doi.org/10.18690/agricultura.16.1-2.35-42.2019

University of Maribor Press

Effects of Oxidation-Reduction Potentials on Soil Microbes

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ABSTRACT

Soil microbes are important in various processes that lead to soil fertility, nutrient availability and plant nutrition. These soil microbial organisms are themselves affected by the environment where they occur. Microbes could either be aerobic or anaerobic depending on their oxygen requirements. Oxidation-reduction reaction is a common reaction in anoxic environments and microbes tend to respond to it in different ways. This study therefore sets out to investigate the effect of oxidation-reduction potentials of the soil on activities of soil microorganisms. Results from this study show that highly reduced soils favors bacteria population more than fungi. It was concluded that the survival of fungi is best supported under oxidized and moderately reduced soils, but their existence can be negatively affected when soils become highly reduced. Bacteria that are aerobic thrive best under oxidized and moderately reduced soil. In these conditions, the highest microbial respiration in the soil was also measured.

Key words: bacteria, fungi, population, respiration, redox potential

INTRODUCTION

The soil houses a large portion of the world's biodiversity and microbes constituting a vast majority of this biodiversity (Menta, 2012). Though the links between soil organisms and functionality are very complex, soil organisms are still considered very important in ecosystems because they affect physical properties and processes like binding together soil particles into aggregates. They also contribute to carbon and energy fluxes, cycling of nutrients as well as soil reactions (Johns, 2017; Kannojia et al., 2019). The chemistry of soil reactions like redox potential (Eh) is regulated by catalytic enzymes that are secreted by soil microbes (Fenchel et al., 2012); so also microbial activities depend on the soil reaction and redox potential (Falkowski et al., 2008). Redox potential has been suggested for characterization of microbial groups, since each type adapted to specific oxido-reducing conditions in either a wide or narrow redox-potential range. Redox potential has an effect on the development of microorganisms. Bacterial growth has been found to be directly correlated to changes in redox potential (Kimbrough et al., 2006). So also, microbial and enzymatic activities are negatively correlated with redox potential in anaerobic soils (Brzezinska, 2004; Kralova et al., 1992). Furthermore, the redox state of nodules is regarded as a referee of legume-rhizobium symbiosis (Marino et al., 2009). The constituents of living organisms particularly proteins are just six elements: oxygen - the strongest oxidizing agent, hydrogen - the strongest reducing agent; and four elements that have the largest amplitude in redox numbers: carbon (–IV in CH_4 to +IV in CO_2), nitrogen (–III in NH_4 to +V in NO_3^-), phosphorus (–III in PH_3 to +V in PO_4^{3-}), and sulfur (–II in H_2S to +VI in SO_4^{2-}). In the process of photosynthesis, plants use light energy to reduce carbon dioxide from the air with hydrogen taken from water (Govindjee and Krogmann, 2004). This reaction produces glucose and releases oxygen. Consequently, the redox state is a critical determinant of the functioning of the cell, and any major imbalances can cause severe damage or death to plants (Dietz and Scheibe, 2004). However, compared with other organisms, soil microorganisms exhibit greater

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Published in 2020

capability of modifying the redox potential and pH of their environment to meet their requirements (Fierer and Jackson, 2006), they can also break down organic matter, making nutrients available for uptake by plants and other organisms and in the process serve as electron shuttle particularly under waterlogged conditions. Decomposition in itself is an oxidation process and is controlled by the interactions of the physical environment and external factors that control the survival and functionality of microbes (Prescott, 2010). The role of the oxidation-reduction potential in determination of the availability of substrates in the surface environment and its essential role in regulation of the abundance and species diversity of microbial communities have been explored by many other authors (Lüdemann et al., 2000, Pett-Ridge and Firestone 2005, Hines et al., 2006). However, updated information is required on the effect of oxidation-reduction status of soil on soil microbes. Hence the objective of this study is to evaluate the effect of redox potential on soil microbial activities.

MATERIALS AND METHODS

The study was conducted using two sandy clay loam soils with different percentages of size fractions. The site of the experiment was at Apatapiti layout around the west gate of Federal University of Technology, Akure (FUTA) Ondo State Nigeria. It lies within the tropical rainforest belt in southwestern Nigeria within latitude 5° 16^{l}N -5° 22^{l}N and longitude 15° 11^{l}E -15° 16^{l}E. An average annual temperature of the location ranged from 21.8 to 32.3 °C. Annual rainfall varied from 1.900 to 2.700 mm, with mean annual evaporation of 2.1 mm. The relative humidity ranged between 85% during the rainy season and less than 60 % during the dry season. The soil according to FAO soil classification is a Nitisol or Alfisol according to USDA classification (FAO, 2006; WRBSR, 2014).

Experimental design and treatments combination:

A 3 × 2 × 2 factorial experiment was set up on two sandy clay loam soils, arranged in a completely randomized design and replicated three times. The treatments combination involved three levels of poultry manure (0 t.ha^{-1}, 6 t.ha^{-1} and 8 t.ha^{-1}), two levels of NPK 15-15-15 (0 kg.ha^{-1}, 200 kg.ha^{-1}) and two watering regimes (field capacity and waterlogging). A total of twelve treatments were obtained with different redox potential (Eh): oxidized soils (Eh > 300 mV), moderately reduced soil (Eh range 100 to 300 mV), reduced soil (Eh range -100 to 100 mV) and highly reduced (Eh < -100 mV).

Soil analysis and sampling

Samples of the soil were randomly collected from the experimental site at a depth of 0-15 cm. The collected samples were analyzed for redox potential and microbial activities from 1 week to 12 weeks after incubation at an interval of 4 weeks (1WAI, 4WAI 8WAI and 12WAI). For analysis of redox potential, a method similar to that described by Rabenhorst et al. (2009) was used. In short, 20 g of the soil samples were collected, soaked in distilled water from bottom to top so as to prevent entrapment of air during saturation and allowed to mix for 30 min after which 50 ml of the solution was collected and taken to the laboratory for reading. In the laboratory, redox potential was measured using a pH/Redox combined meter. Voltage was measured every 10 s for 60 s and the mean values of the collected measurements were calculated.

Soil microbial population was carried out using the colony count method. Soil samples were collected and stored in a sterile container for cultivatable fungal and bacterial analysis. Serial dilutions (up to 10^{-5}) were prepared and 1 ml from each dilution was dispensed on nutrient agar for the culture of bacterial while potato dextrose agar was used for fungal culture. Bacteria culture was done according to the description given by Paul and Clark (1989). Inoculated plates were incubated at 25 °C for 48 hours after which the colony formed were counted. Culture of fungi was done following the procedure of Harrigan and McCance (1976). The inoculated plates were incubated for 5 days at a temperature of 28 °C and total number of fungi was counted. Number of colony for each dilution was counted and the amount of bacteria and fungi were calculated by using the following equation:

CFU/ml = (number of colonies × dilution factor) / (volume of cultured plate)

Gas entrapment method was used to determine the microbial respiration of the soil samples in this study. 10 ml of 0.5 M NaOH was dispensed into a beaker and placed on the top of 50 g soil inside a plastic jar to trap CO_2 evolved from the soil. A separate jar without soil was used as a control which also contained 10 ml of 0.5 M NaOH to account for CO_2 trapped from the atmosphere. After 24 hours, the NaOH was gently removed, covered with an aluminum foil and immediately passed on for CO_2 determination. 1 M $BaCl_2$ was added to the NaOH beaker to precipitate the carbonates to facilitate determination of CO_2 evolved from the soil after which the evolved CO_2 was determined by titration. Excess NaOH was titrated against 0.5 M HCl using phenolphthalein as indicator after precipitating the carbonate formed with 1.0 M $BaCl_2$. The solution goes from pink to white and then colorless which represents the end point. CO_2 was then calculated using the following formula:

$CO_2 = (B \times V) \times (N \times E)$
where:
B= blank (reading of the CO_2 trapped from the atmosphere (mol^{-1}))
V= volume of acid (final value – initial value (mol/L))
N= normality of acid (meq/L)
E= equivalent weight of C in CO_2 (g)

Data Analysis

Data obtained from this study were subjected to analysis of variance (ANOVA) using statistical package SPSS version 17,

while means where separated using Tukey Range test. Figures were generated using MS excel 2010 to show the trends of findings.

RESULTS

Table 1 presents the pre-experiment analysis of soils; the pH was measured in water. Soil 1 was a slightly acidic (pH = 6.26) sandy clay loam, having a sand content of 54.8%, silt of 20.5% and clay of 24.7%. Its redox potential was 260 mV making it a moderately reduced soil, bacteria population of 3.70×10^{-5} cfu.ml^{-1} and fungi population of 2.66×10^{-5} cfu.ml^{-1}. Soil 2 was a slightly alkaline (pH = 8.25) clay loam having a sand content of 48.0%, silt content of 21.4% and clay content of 30.6%. It was also a moderately reduced soil with Eh of 282 mV. Bacteria population of soil 2 was 4.76×10^{-5} cfu.ml^{-1} and fungi population of 3.41×10^{-5} cfu.ml^{-1}. Generally, the two soils are considered moderately fertile.

Table 1: Pre-experimental soil analysis

Properties	Soil 1	Soil 2
Redox potential (mV)	260	282
pH (water)	6.26	8.25
Sand %	54.8	48.0
Silt %	20.5	21.4
Clay %	24.7	30.6
Textural class	Sandy clay loam	Sandy clay loam
Bacteria population (cfu.ml^{-1})	3.90×10^{-5}	4.76×10^{-5}
Fungi population (cfu.ml^{-1})	2.66×10^{-5}	3.41×10^{-5}
Microbial respiration (mg CO_2-C.g^{-1}h^{-1})	13.5	15.6
Organic matter %	1.60	2.36
N %	0.19	0.17
P (ppm)	15.2	24.8
K (cmol.kg^{-1})	0.16	0.18

Table 2 shows the classification of treatments based on redox potential (Eh) values. T1 (200 to 370 mV), T3 (260 to 430 mV) and T7 (280 to 310 mV) fell into oxidized soil (Eh > 300 mV), T5 (200 to 290 mV), T9 (230 to 300 mV) and T11 (230 to 300 mV) were moderately reduced (100 mV to 300 mV), T2 (-120 to -100 mV) and T4 (-130 to -100) were reduced soils (-100 mV to 100 mV) and T6 (-240 to -210 mV), T8 (-230 to -200 mV), T10 (-240 to -230 mV) and T12 (-240 to -230 mV) were highly reduced soils (< -100 mV).

Table 3 presents the effect of redox potential on soil biological properties (bacteria population, fungi population, basal and substrate induced respiration) at 12 weeks after incubation (WAI). On soil 1, highly reduced soils and moderately reduced soils {T12 (4.31×10^{-5} cfu.ml^{-1}), T11 (4.08×10^{-5} cfu.ml^{-1}), T9 (4.08×10^{-5} cfu.ml^{-1}), T8 (4.29×10^{-5} cfu.ml^{-1}), T7 (4.03×10^{-5} cfu.ml^{-1}) and T6 (4.04×10^{-5} cfu.ml^{-1})} recorded the highest bacteria population, however no significant difference was observed among them. The lowest bacteria population was observed on T1 (2.87×10^{-5} cfu.ml^{-1}), T2 (2.67×10^{-5} cfu.ml^{-1}) and T4 (2.32×10^{-5} cfu.ml^{-1}) (oxidized soil and reduced soil). Fungi population was the highest on moderately reduced soil T11 (4.22×10^{-5} cfu.ml^{-1}) and the lowest on highly reduced soil T12 (2.54×10^{-5} cfu.ml^{-1}). CO_2 content for basal respiration was the highest on T9 (moderately reduced) with value 103.2×10^{-5} cfu.ml^{-1}, the lowest on T1 (oxidized) with value 13.7×10^{-5} cfu.ml^{-1}, while substrate induced respiration was highest on T5 (moderately reduced) with value 34.8×10^{-5} cfu.ml^{-1} and lowest on T1 (oxidized) with value 12.0×10^{-5} cfu.ml^{-1}. The same trend was observed in soil 2.

Figures 1 and 2 presents the microbial population trend of bacteria and fungi, respectively. Figures show the trend of the microbial population over incubation time as affected by the redox potentials. In all, there are four redox potentials and the four are represented in the graph. The twelve treatments fall into one of the redox potential represented on the graph.

Figure 1 shows the bacteria population trend from 1WAI to 12WAI for the two soils used for the trials. Black line represents soils that were subjected to highly reduced conditions, red line represents reduced soils, green represents moderately reduced and blue represents oxidized soils. Similar trend

Table 2: Classification of treatment based on redox potential (Eh) range

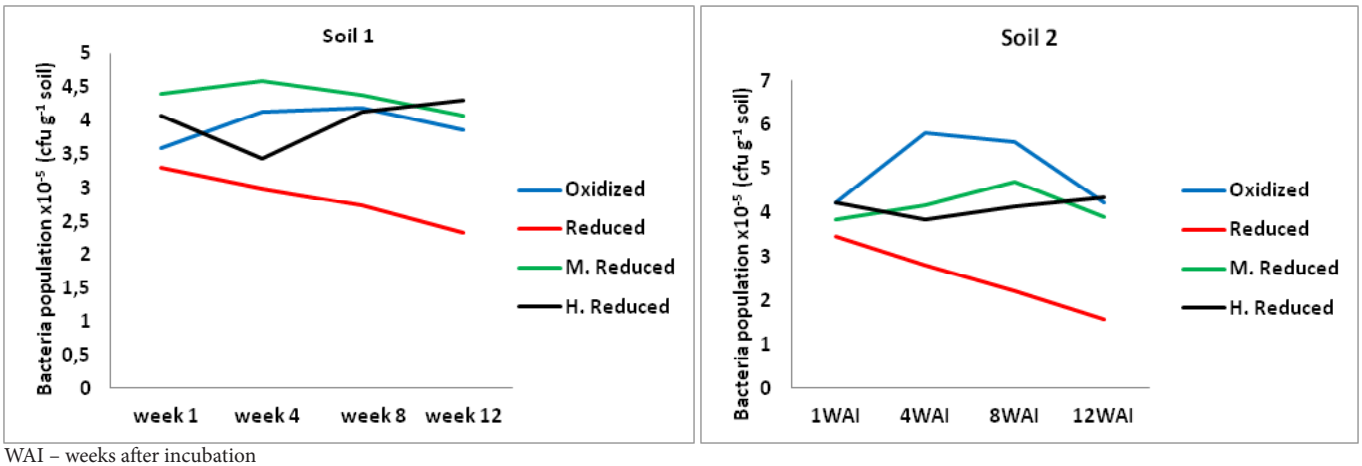
Treatments	Treatment description	Eh range (mV)	Eh class
T_1	0 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	200 to 370	Oxidized
T_2	0 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-120 to -100	Reduced
T_3	0 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	260 to 430	Oxidized
T_4	0 t ha^{-1} PM + 200 kgha^{-1} NPK + WL	-130 to -100	Reduced
T_5	6 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	200 to 290	Moderately reduced
T_6	6 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-240 to -210	Highly reduced
T_7	6 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	280 to 310	Oxidized
T_8	6 t ha^{-1} PM +200 kg ha^{-1} NPK + WL	-230 to -200	Highly reduced
T_9	8 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	230 to 300	Moderately reduced
T_{10}	8 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-240 to -230	Highly reduced
T_{11}	8 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	230 to 300	Moderately reduced
T_{12}	8 t ha^{-1} PM + 200 kg ha^{-1} NPK+ WL	-240 to -230	Highly reduced

PM – Poultry manure, NPK – NPK 15-15-15, WL - Waterlogged, FC – Field capacity, Eh – redox potential

Table 3: Effect of redox potential on biological properties of soil

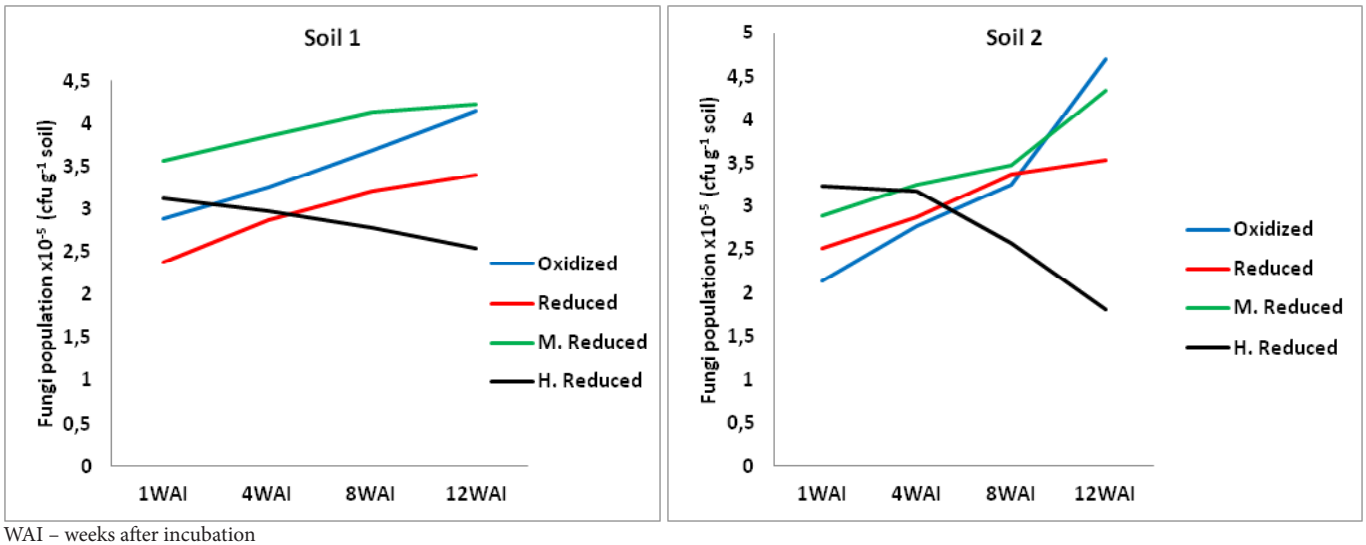
Treatments	Soil 1				Soil 2			
	Bacteria	Fungi	BR	SIR	Bacteria	Fungi	BR	SIR
	cfu ml^{-1} soil × 10^{-5}		mg CO_2-C $g^{-1}h^{-1}$		cfu ml^{-1} soil × 10^{-5}		mg CO_2-C $g^{-1}h^{-1}$	
T_1	2.87c	3.49ab	13.7i	12.0g	1.42e	3.90abc	42.6g	10.2g
T_2	2.67c	3.40b	7.80j	15.0f	1.66e	3.41c	31.8h	12.6f
T_3	3.86b	4.14ab	74.6f	32.4b	4.21abc	4.70a	64.6e	27.2c
T_4	2.32c	3.40b	28.8h	26.4d	1.57e	3.54bc	61.0f	22.7e
T_5	3.74b	3.45ab	76.8e	34.8a	4.43ab	4.04abc	73.6c	27.7c
T_6	4.04a	1.65d	54.4g	30.6c	3.63bcd	1.11e	65.9e	25.4d
T_7	4.03a	3.61ab	81.6c	34.2a	3.49cd	4.19abc	75.6b	32.1a
T_8	4.29a	2.43cd	78.0d	23.4e	3.93a-d	1.41e	76.1b	29.2b
T_9	4.08a	3.89ab	103.2a	32.4b	3.32d	4.13abc	105.8a	31.8a
T_{10}	3.85b	2.35cd	76.8e	12.0g	3.82bcd	2.45d	73.1c	28.9b
T_{11}	4.08a	4.22a	89.4b	10.2h	3.88a-d	4.33ab	76.2b	29.4b
T_{12}	4.31a	2.54c	76.8e	25.8d	4.71a	1.81de	70.3d	12.6f

Means followed by the same letters are not significantly (p>0.05) different according to Tukey HSD.
BR – Basal respiration, SIR – Substrate induced respiration



WAI – weeks after incubation

Figure 1: Bacteria population trend from 1WAI to 12 WAI for both soil types



WAI – weeks after incubation

Figure 2: Fungi population trend from 1WAI to 12 WAI for both soil types

was observed on treatments with the same redox status. Bacteria population increased from 1WAI to 4WAI for most soils that were subjected to moderately reduced and oxidized conditions, the population status was maintained up to 8WAI, but declined at 12WAI. There was a continuous decline in bacteria population from 1WAI to 12WAI under reduced condition. Under highly reduced condition, there was a sharp decline in bacteria population from 1WAI to 4WAI which later picked up from 4WAI to 8WAI and was maintained till 12WAI. Figure 2 shows the fungi population trend from 1WAI to 12WAI for the two sites used for the trial. Black line represents soils that were subjected to highly reduced conditions, red line represents reduced soils, green represents moderately reduced and blue represents oxidized soils. Soils subjected to highly reduced condition were observed to have a continuous decline in fungi population from 1WAI to 12WAI, oxidized and moderately reduced soil had a constant increase in fungi population from 1WAI to 12WAI. The same trend was observed in soil 2. Generally, bacteria population density from 1WAI to 12 WAI in soil 1 ranged from 2.0×10^{-5} to 4.5×10^{-5} for all redox potential status, while soil 2 ranged from 1.8×10^{-5} to 5.9×10^{-5} with moderately reduced soil having the highest in soil 1 and oxidized in soil 2, reduced soil was lowest in both cases. Fungi population density from 1WAI to 12WAI ranged from 2.3×10^{-5} to 4.0×10^{-5} for soil 1, while soil 2 ranged from 1.8×10^{-5} to 4.6×10^{-5} for soil 2. Highly reduced soils recorded the lowest values while moderately reduced soil had the highest in soil 1 and oxidized soil in soil 2.

Results from this research showed that fungi population and their survival is best under an oxidation-reduction potential of 100 mV to > 300 mV (moderately reduced and oxidized). Most fungi, particularly those involved in agricultural processes, are known to be obligate aerobic organism and as such require packets of oxygen for survival. This agrees with the statement of Magdoff and Van Es (2012) that under compacted soils bacteria can outdo fungi because of low level of oxygen. Given that reduced and highly reduced conditions have no or low oxygen, they cannot thrive well under such condition. Aerobic bacteria also require oxygen to live and cannot survive under reduced or anoxic conditions, hence, the continuous increase in population from 1WAI to 8WAI under oxidized soils (Fig. 1). According to a research by Hibbing et al. (2010), at the peak of their life cycle, bacteria develop survival mechanism sometimes through the release of toxins into the environment. This coupled with increased competition for nutrient (Amarasekare, 2002), available oxygen (Riedel et al., 2013) and space (Lloyd and Allen, 2015) when population becomes dense can cause microbial population to drop. This could account for the decline in the population observed at 12WAI. When an environment is subjected to waterlogging, oxygen is expelled; this triggers a change in the type of organism that can survive under that condition. In a report by Stamati et al. (2011) on the effect of oxygen on evolution, it is stated that although oxygen is not the only factor that can trigger evolution, however, fluctuation of oxygen can determine the survival and changes of organism in an environment. At the point of low oxygen content in the soil, facultative anaerobe starts taking over the environment and gradually to obligate anaerobes. This phenomenon could be responsible for the sharp decline in population between 1WAI to 4WAI for highly reduced soil which later increased at 8WAI. At 1WAI most of the bacteria are aerobic, but could not survive till 4WAI, because of the anoxic condition of the soil. At 4WAI facultative anaerobe began to take over up to 8WAI, then couple with obligate anaerobes increased the population at 12WAI. This corresponds with an article by Chu (2016) who reported that in the study of marine science greater population of bacteria that are active where reduction process is more pronounced are facultative and obligate anaerobes. However, these obligate anaerobes cannot survive under oxic conditions because oxygen present in air act as excited singlet oxygen which can react with water in their cell to form hydrogen peroxide which is highly reactive and lethal to them because they do not possess any defense for these peroxides. Hence, the continuous increase in bacteria population under oxidized conditions can be attributed to the fact that throughout the trial period, the predominant bacteria were aerobic bacteria. Aerobic organisms, breakdown carbohydrate to form glucose and much energy are released. Under reduced conditions, prevailing organisms can split glucose into just two molecules of pyruvate, however, under oxidized conditions prevailing organisms can breakdown glucose all the way to carbon dioxide which is given off as waste product during cellular respiration. This could account for the high amount of CO2 that is present in oxidized soils compared with reduced and highly reduced soils.

Comparatively, fungi sustainability under highly reduced soils is not affected by the soil texture, that is the percentage of sand or clay in the soil does not contribute to the overall effect of treatments on fungi. This could be attributed to the fact that most fungi are aerobic in nature and reduced conditions will deprive them of oxygen. The work by Magdoff and Van Es (2012) also supports this claim stating that bacteria can perform better than fungi under low oxygen conditions. However, soil 2 has larger population of fungi than soil 1 for both moderately reduced and oxidized soils. According to an article by the Department of Agriculture of the Republic of South Africa in 2007, soils with more clay content are generally more fertile than those with more sand, this phenomenon according to Malavolta (1980) occurs because of their ability to absorb ion from solution. A report by Hayman (1982) indicated that high soil fertility can support fungi growth and survival as well as benefits mycorrhizal fungi in particular. Bacteria populations under highly reduced and reduced soils follow the same density for soil 1 and 2. This shows that under these two redox potential conditions, the sand or clay content or texture of the soil does not contribute to the resulting bacteria population density. However, soil 2 has more bacteria density than soil 1 under moderately reduced and oxidized conditions; this can be linked to the fertility and water holding status of individual soils. Works by Garbeva et al. (2004) and Fang et al., (2005) showed that soil type influenced microbial communities' particularly bacterial population. Najmuldeen (2010) also found out that clay loam and silt clay loam had a greater population of bacteria than sandy loam. This could be due to high nutrient and moisture content of soils with more clay content. Heritage et al. (2003) explained that sandy soils cannot retain water and can easily drain out while clay soils have better water and nutrient holding capacity.

CONCLUSION

The oxidation-reduction potential of soils determines to a large extent the presence or absence of soil microbes particularly the types that will be prevalent in the soil and their functionality. Based on results from this research, the survival of fungi is best supported under oxidized and moderately reduced soils, but their existence can be negatively affected when soils become highly reduced. Bacteria that are aerobic thrive best under oxidized and moderately reduced soil. However, certain bacteria that are anaerobic in nature thrive best under highly reduced conditions. Given that the trend lines of the two soils used, though similar but not consistent as regards the values, it can be concluded that texture of the soil with oxidation-reduction potential that is greater than 100 mV (moderately reduced and oxidized) contributes to the response of microbes to oxidation-reduction status of soil. However, when oxidation-reduction potential is less than 100 mV (reduced and highly reduced), the effect of soil texture is not significant. However, it is recommended that this trial be repeated on the other soil textures.

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Declaration of Interest Statement

I declare that there is no conflict of interest of any kind between the authors of this manuscript.

Učinek oksidacijsko-redukcijskega potenciala na talne mikrobe

IZVLEČEK

Talni mikrobi so pomembni pri različnih procesih, ki prispevajo k rodovitnosti tal, razpoložljivosti hranil in prehrani rastlin. Delovanje talnih mikroorganizmov je odvisno od okolja, v katerem živijo. Mikrobi so lahko aerobni ali anaerobni, odvisno od njihovih potreb po kisiku. Oksidoredukcijske reakcije so v anoksičnih okoljih pogoste in mikrobi se nanje odzivajo na različne načine. Cilj te raziskave je preučiti vpliv oksidacijsko-redukcijskega potenciala tal na aktivnost talnih mikroorganizmov. Rezultati raziskave so pokazali, da so močno reducirana tla bolj ugodna za razvoj populacij bakterij kot pa populacij gliv. Ugotovljeno je bilo, da so za preživetje gliv najbolj ugodna oksidirana in zmerno reducirana tla, medtem ko lahko močno reducirana tla negativno vplivajo na njihov obstoj. Aerobne bakterije uspevajo najbolje v oksidiranih in zmerno reduciranih tleh. V teh pogojih so bile izmerjene tudi najvišje vrednosti mikrobnega dihanja v tleh.

Ključne besede: bakterije, glive, populacija, dihanje, redoks potencial