IMPROVED MANAGEMENT OF ACID SULFATE SOILS FOR RICE PRODUCTION IN CASAMANCE, SENEGAL

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ABSTRACT

Casamance is a region in southern Senegal that traditionally produces rainfed rice, but Senegal produces only 1/3 of its rice consumption. Lowland areas, where rice is primarily produced, have acid sulfate soils with low pH and potential aluminum and iron toxicity. The goal of this work was to determine if soil amendments can alleviate soil acidity, counteract the negative biogeochemical effects that occur in flooded conditions, and increase rice yield. A two-year experiment was conducted to test the following soil treatments – agricultural lime, pulverized oyster shell, biochar, and control (no amendment) – in flat and raised beds. Plots amended with lime and shell materials had increased soil pH, base saturation, Ca, and cation exchange capacity. Meanwhile, biochar elevated particulate organic matter and C:N ratios. Exchangeable Fe and Al were negatively correlated with soil pH, while *Geobacteraceae* populations (Fe reducing bacteria) increased with pH. A greater proportion of the total Fe was strongly bound in fractions that were less bioavailable in plots amended with shell or lime, and overall rice yields were significantly higher following amendment with shell or lime. During the second growing year these effects diminished, suggesting that liming effects did not persist as expected. These results demonstrate the benefits of soil amendments that raise soil pH and suggest that this effect operates by influencing overall soil nutrient availability to rice plants, but further research is needed regarding the timing and sustainability of the beneficial liming effect.

Improved Management Of Acid Sulfate Soils For Rice Production In Casamance, Senegal

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GENERAL AUDIENCE ABSTRACT

Casamance is a region in southern Senegal that traditionally produces rainfed rice, a primary cereal for human consumption. However, Senegal produces only 1/3 of its rice consumption. In addition to record rainfall deficits in recent years, degraded soils in lowland areas where most rice is produced are characterized as acid sulfate type. These soils, at acid pH, produce toxic amounts of soluble ions that reduce rice yields and amplify deficiencies of other essential nutrients. Furthermore, these conditions reduce the amount of lowland field that can be cultivated. The objective of this work was to increase rice yield in Casamance lowland through the use of soil amendments to alleviate soil acidity and the compounding negative effects that occur in flooded conditions. A two-year experiment was established to test the following soil treatments – agricultural lime (13 t ha^{-1}), pulverized oyster shell (13 t ha^{-1}), biochar (15 t ha^{-1}), and control (no amendment) – in two different planting systems, flat and raised beds. Chemical and biological characteristics were analyzed at four different times during the growing season including transplantation, panicle initiation, flowering, and harvesting. Agronomic data were also collected to quantify improved fertility and rice productivity. Results from the first year showed increased soil pH and decreased iron concentrations in experimental plots amended with lime and shell. The biochar treatment had the highest particulate organic matter and C:N ratio. Exchangeable Al^{3+} in the soil was significantly and negatively correlated with soil pH. Exchangeable iron in the soil decreased following amendment with shell and lime and a strong relationship was detected between exchangeable iron and total iron concentrations. *Geobacteraceae* populations (iron reducing bacteria) increased with pH. Following sequential extraction of various iron fractions, soil amended with shell or lime also contained a greater proportion of total iron in strongly bound fractions that are less plant available. Rice plant growth and yield parameters were also significantly higher in plots amended with shell and lime. For the second growing season these effects decreased in all treatments, suggesting that the liming effect does not last as long as observed in other cropping systems and soil types. The results suggest that the benefit of soil amendments that raise soil pH in these areas operate by influencing the soil nutrient availability to rice plants, but further research should be done regarding the timing and sustainability of the beneficial liming effect. Results of this work contribute to a better understanding of the Casamance lowland ecosystem and will hopefully help recover currently unproductive lands that can help meet the food demands of the Senegalese population.

Dedicated to
My late Mum Katy, my friend
My late Dad Doudou, my example
With appreciation for their love and dignity

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1. INTRODUCTION

In Senegal, rice is primarily cultivated in the north along the Senegal River, in the center of the country, and in Casamance in the south. Rice fields in the north are mostly irrigated while the central and southern crops are rainfed. Currently, Senegalese domestic rice production covers only 40 % of population needs (Demont and Rizotto, 2012). To meet food demands, improvement and rehabilitation of paddy fields with specific strategies for each region are necessary. One challenge is that the southern region of Casamance, where rice is the primary cultivated cereal, has recorded rainfall deficits in recent years (Thiam and Singh, 2002). In addition, soils in the region have a variety of physical and chemical characteristics that stress rice plants and reduce the amount of lowland fields that can be cultivated (Sambou et al., 2015). One of those stresses is iron (Fe) toxicity, which is prevalent in the lowland areas that produce the majority of rice and which results from the acid sulfate soil type that is common in the region.

Sulfidic materials form naturally where sulfate rich materials such as sea water mix with materials containing iron and organic matter in a waterlogged or highly reduced environment (i.e., mangrove sediments) and form metal sulfides, predominantly pyrite (FeS_2). Subsequent exposure to oxygen by drainage or excavation leads to oxidation of the precipitated sulfides and sulfuric acid production. The resulting lower pH plus high concentrations of soluble aluminum (Al) and Fe limit the productivity of acid sulfate soils unless these deleterious effects can be ameliorated using appropriate agronomic practices (Shazana et al., 2013).

Chemical and biological processes in acid sulfate soils produce large amounts of soluble Fe during seasonal flooding, which, coupled with water flow from uplands to lowlands, results in toxic levels of dissolved Fe in lowland fields. Iron toxicity can reduce rice yields by 12-100% (Sahrawat, 2004), depending on the intensity of the stress and the tolerance of rice cultivars. This effect is further amplified by deficiencies in other essential elements required for the growth of the plant (Fageria et al., 2008). Studies suggest that lime, tolerant rice varieties, and water management are the most promising ways to minimize Fe toxicity (Shazana et al., 2013; Audebert and Sahrawat, 2000; Matthew et al., 2001). While Fe tolerant varieties are an important strategy for increasing rice yields in Senegal, the tolerant varieties are still overridden by adverse soil properties. Improved soil management, therefore, represents an important component of an overall strategy for improving rice yield.

Iron toxicity is a significant barrier to increasing rice yields (Sahrawat, 2004) and reaching the food security objectives in Senegal. Furthermore, the soil is a dynamic system and Fe affected lands have increased over the years in Senegal, which has further reduced usable arable land area. This challenge can be only solved with proper management of multiple components of the total agroecosystem. However, the understanding of the complex dynamics in these systems that is required to design management practices to recover unproductive lands is currently lacking. Moreover, available data from research on this problem in the Casamance region are limited and date back to the period of 1967-1986.

The dynamics of Fe-toxicity are a complex mix of physics, chemistry, and biology, with rapid changes in soil conditions expected during the course of a growing season as water levels and oxygen availability in the soil change (Moore and Patrick, 1989). This study was designed to address the critical knowledge gap about the ability of soil amendments to mitigate Fe-toxicity, increase rice yield, and improve soil fertility in Casamance lowlands. Specifically, the following objectives were addressed:

1. Quantify the ability of locally available soil amendments to improve soil conditions;
2. Determine the overall effects of soil amendments on Fe availability and toxicity;
3. Measure the effects of soil amendments on rice yield.

The tested hypotheses were:

1. Amending soils with limning material will raise soil pH during the rice growing season.
2. Amended soils with increased pH will result in decreased concentrations of soluble Fe and Al.
3. Amended soils with increased pH will result in higher nutrient availability to rice plants and increased crop yields.

The manuscript is organized into four chapters. The first chapter is a literature review and the materials and methods used for the laboratory and experimental research are presented in the second chapter. Results and data are presented in the third chapter and the fourth chapter interprets the results and discusses their significance.

2. LITERATURE REVIEW

Rice is one of the most widely grown and consumed cereals in the world with 159 M ha cultivated (FAO, 2008), mainly in tropical zones. It is an annual grass that has a life cycle from germination to maturity that varies based on the variety and the environment. Jia-fang and Shi-ye (1981) define high fertility paddy soils as having 2-4% organic matter and 0.13-0.23% nitrogen, as well as total phosphorus (P) and potassium (K) contents over 0.1% and 1.5%, respectively. Furthermore, the soil should be characterized by a large buffering capacity, proper permeability with good structure and capillary porosity, and being devoid of toxic levels of substances.

The main cultivated rice species are *Oryza sativa*, which originated from Asia, and *O. glaberrima* from Africa. Growing practices are dependent upon the water regime; upland rice is defined as rice grown without flooding, usually on hillsides, while lowland rice is grown in fields that can be naturally flooded by rainfall or irrigation. Water depth and timing are variable because of seasonal irrigation and drainage practices. The variability between flooded and non-flooded periods is critical, mainly because the associated changes in oxygen availability in the paddy soil control important redox reactions and pH changes (Ponnamperuma, 1972; Konsten et al., 1994; Shamsuddin et al., 2013). To survive in the reducing environment, which results from the decreased level of oxygen during flooding, rice plants develop aerenchyma cells in the roots that provide oxygen exchange with the atmosphere. In Casamance, rice varieties from *O. sativa* are most often cultivated in lowland areas. Rice farming systems are adapted to local ecological conditions and extend from the uplands to the inland valley or the mangrove swamps. Thus, upland rice varieties are distinguished by short life cycles. The submerged freshwater rice is cultivated in the valley and often records the highest yields in lower Casamance. In contrast, rice flooded with salt water is grown in the mangrove areas with salt tolerant varieties.

Like most of the working population of Senegal, where 70% of the total population is involved in agriculture, farming is the main livelihood for residents of Casamance and especially in Ziguinchor (Osameka, 2011). The principal crops are groundnuts, rice, millet/sorghum, and maize, but agricultural activity is dominated by rice cultivation. It is a self-sufficient activity conducted mainly by women at all stages including the nursery, rice transplanting in the lowland and mangrove areas, harvesting, mowing, and husking. Men, however, are responsible for land preparation and plowing and the establishment of ridges in submerged areas. The agronomic practices are still traditional, with limited use of chemical fertilizers or soil amendments and low yields.

Globally, rice production is often less than the demand and this is particularly true in Africa (Sasson, 2012). This problem is compounded by recent rainfall deficits in regions where rice is cultivated. In addition, soils in the region have a variety of physical and chemical characteristics that stress rice plants and reduce the amount of fields that can be cultivated. Iron toxicity is one of the stresses particularly prevalent in the lowland areas that produce the majority of rice in Casamance and results from the acid sulfate soil type that is common in the region. Rice varieties have varying degrees of adaptation to Fe-toxicity (Audebert and Sahrawat, 2000; Becker and Asch, 2005), but the ability to manage soils directly to reduce Fe-toxicity would be a valuable tool for improving rice yields in Senegal.

2.1 Acid Sulfate Soils

2.1.1 Formation and origin

Acid sulfate (AS) soils have a worldwide distribution (Dent and Pons, 1995) and occur in inland areas such as wetlands, mine sites, and lakes as well as coastal mangrove areas. The pedogenesis of these soils is dominated by sulfur (S) being present in original form as sulfides (H_2S, S°) and soil horizons that contain sulfides are called sulfidic materials (Soil Survey Staff, 2003). The waterlogging and reducing conditions of the flooded environments where AS soils mostly exist are favorable for the formation of sulfide minerals. Formation and oxidation of metal sulfides are important parts of the geochemical cycles (Fe and S) in these soils. Depending on redox conditions, these sulfides exist in stable reduced forms or in oxidized forms, which differentiate between potential and active acid sulfate soils, respectively. Sulfide accumulation is promoted by sufficient sources of S and Fe along with organic matter that, in a reducing environment, favors anaerobic respiration by sulfate-reducing bacteria that catalyze the reaction. Specifically, sulfate reducing bacteria reduce sulfate in a dissimilatory manner during the anaerobic degradation of organic matter and produce hydrogen sulfides (Holmer and Stockholm, 2001).

These hydrogen sulfides may then further react with Fe in the reducing environment. In the monosulfidic reaction, Fe ions (Fe^{2+}) react with sulfide:

$H_2S+Fe \rightarrow FeS+H_2O$ (Equation 1)

A disulfidic reaction may also occur where Fe reacts with sulfide in the following manner (Golez, 1995):

$FeS+S \rightarrow FeS_2$ or $FeS+H_2S \rightarrow FeS_2+H_2O$ (Equation 2).

Overall, FeS_2, commonly called pyrite, is the most common sulfide observed in acid sulfate soils (Bush et al., 2000). It is harmless to plants in this fully reduced state, but may oxidize when exposed to air and water, re-release metals and sulfate, and decrease pH via the production of H_2SO_4 (Dent, 1986):

$FeS_2 + 3^{1/2}O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4$ (Equation 3)

Decreases in pH can be below 4 in such scenarios due to the fact that buffering capacity is exceeded (Ljung et al. 2009) and mainly because Fe oxidizing bacteria (*Thiobacillus ferrooxidans*) catalyze the reaction (Singer and Stumm, 1970; Nordstrom, 1982). *T. Ferrooxidans* obtains its energy from the oxidation of ferrous to ferric, reduces forms of sulfur and metal sulfides and assimilates CO_2 by chemosynthesis. Their activity regenerates the supply of ferric irons in solution (Nordstrom, 1982). So, pyrite can be reformed and re-oxidized under dry period leading to higher acidity during the next rainfall. The ferrous Fe sulfate in equation (3) can be further oxidized to ferric Fe (Fe^{3+}). So, at pH<4 and a strongly reduced environment, ferric iron can be reduced again to ferrous iron:

$FeS_2+2Fe^{3+} \rightarrow 3Fe^{2+}+2S$ (Equation 4)

And at pH>4, ferric Fe reacts with water to hydrolyze and precipitate as ferric hydroxide, generating substantially more acidity:

$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$ (Equation 5)

The generated acidity is retroactive. Also, it is often advocated to maintain the sulfidic sub-soil in a flooded state to avoid acid generation (Sahrawat, 1979). The initiation of the oxidation process is generally related to a lowering of the water table and artificial drainage for agricultural purposes, dredging, mining, or other excavation that greatly accelerates and compounds the problem (Orndorff and Daniels, 2004).

2.1.2 History and geology of acid sulfate soils in Senegal

In Senegal, soil science research intensified in the early 1970's when soil acidification became acute in crop production areas, particularly in rice fields (Marius, 1979). The low pH was found to be caused by the prevalence of acid sulfate soils in tidal areas (Beye, 1973; Marius, 1979; Sadio and van Mensvoort, 1993). This acidification resulted from the catastrophic drought of the 1970s and the drop of the groundwater table, causing pyrite oxidation of the marine sediments. The failure of the reclamation of mangrove swamp soils was due to the inadequacy and the irregularity of the rains for proper leaching of toxic substances and the instability of chemical reactions driven by pyrite oxidation that led to the development of acid sulfate conditions (Toure, 1981).

In Casamance (southern Senegal), sediments that formed from Holocene deposits were derived from the plateau soils of the Continental Terminal formation of Casamance, which are rich in quartz, kaolinite clays and Fe oxides (Viellefon, 1977). They were deposited under the influence of marine intrusion responsible for a regular deposit of S rich sediments. Subsequent establishment of mangroves such as *Rhizophora* or *Avicennia* in tidal areas contributed organic matter via root production and exudation. Acid sulfate soils contained various sulfate minerals whose spatial distribution was described in the Djiguinoum valley, Lower Casamance (Montoroi, 1995). In central Senegal, the topography is considered as the main factor that controls intrapedon processes such as gleyzation, sulfidicization and sulfuricization, as well as interpedon processes such as salinization, colluvio-alluviation and lateral eluviation (Aidara et al., 2014). In the Senegal valley (northern Senegal) acid sulfate soils are found in the delta fossil soils buried under recent sediments (Deckers et al.,1993; Barbiero et al., 2005).

2.1.3 Effects of acid sulfate soils on agronomic systems

Under normal circumstances, acid sulfate soils cannot be productively utilized for agriculture. Many crops are intolerant of swampy conditions and soil acidity, which is why rice is often the first crop to be considered in such areas due to its tolerance of these conditions. However, the pH tolerance of rice does not solve the entire problem. Concurrent reactions producing soluble Al, Mn, and high concentrations of ferrous sulfate may occur when the pH decreases subsequently under submerged conditions ($pH < 4$) (Shamshuddin et al., 2013). In the soil profile, the depth and extension of oxidation depend on drainage and soil aeration, so O_2 and pH variations are the main factors that affect the vertical location of the oxidation process along the soil profile. Iron resulting from oxidation of pyrite is increased by the interactions between water regime and topography favoring the flow of Fe mineral fractions (ferric and ferrous forms) through the landscapes (Husson et al., 2000). When fields are re-flooded during the rainy season for rice production, oxygen is rapidly depleted again by heterotrophic microbial activity. Eventually, anaerobic Fe-reducing bacteria become active in the soils and produce large amounts of reduced Fe (Fe^{2+}), which is toxic to rice plants at the low pH values (<3.5) at which Fe is typically quite soluble (Weber et al., 2006).

The forms of Fe present in soil depend on the redox conditions and the soil pH. In dry aerobic conditions, the oxidized form (Fe^{3+}) dominates the Fe pool, while in saturated anaerobic conditions the reduced form (Fe^{2+}) is dominant. The main sources of Fe are the soil minerals and organic matter, and in saturated zones, O_2 is rapidly depleted by aerobic microorganisms during organic matter degradation. Obligate and facultative anaerobic organisms further decompose organic matter using oxidized soil components as electron acceptors (Fisher, 1988). Nitrate is reduced to gaseous forms through denitrification (Carrasco et al., 2004) Mn, Fe, sulfate reduction follow, and finally methanogenesis occurs (Yao et al., 1999; Conrad, 2007). High amounts of anaerobic activity in these soils are key because the availability of alternative electron acceptors are the primary cause of the active cycling of Fe and S that form the foundation of Fe-toxicity. This causes the physiological nutrient disorder in rice plants that underlies the effects of Fe-toxicity in acid sulfate soils. Therefore, in addition to tolerant rice varieties, yield increases will require improved practices using appropriate water management strategies and soil amendments (Ai et al., 2015).

2.2 Nutrient toxicity: Iron toxicity

Iron toxicity is one of the main nutrient disorders in lowland areas that affects rice farming (Sahrawat, 2004).A concentration of 300 mg/L of soluble Fe was reported by Lantin and Neue (1989) and Elec et al. (2013) to be the critical limit for the cultivation of lowland rice. Variability is often globally observed around this threshold based on the type of soil (Fageria et al., 2008). Given that Fe is a micronutrient, some Fe is required for healthy plant growth, and toxicity only arises with high concentrations of soluble Fe. If present in abnormally high amounts in plant tissues, it can negatively affect plant growth, with symptoms among affected plants that include chlorosis and necrotic spots on the lower leaves and brown roots (Fageria et al., 2008). Reduction in plant height, number of tillers, and seedling emergence have also be observed (Hagiwara and Imura, 1993).

Iron concentrations in lowland soils are the result of three interrelated factors: local Fe content within the acid sulfate soils, overland flow or surface runoff of soluble Fe from upland to lowland, and biological activity of Fe reducing bacteria. Given these complex dynamics, Fe toxicity can be highly variable in time and space. Prade et al (1986) observed two peaks of intensive Fe (II) formation and uptake in Casamance: one during the first week after rice transplantation (which they termed primary Fe toxicity) and the second between heading and flowering (termed secondary Fe toxicity). They related the primary toxicity to the sensitivity of freshly transplanted rice seedlings and the impacts of toxicity at this stage can affect plant growth and result in a complete yield loss (Abifarin, 1988). The secondary period is related to a deficiency of K, which is in high demand during that stage of plant growth. There is an antagonistic interaction for rice plants between K and Fe occurring either outside the root or within the root that Fageria et al. (1981) reported with the increasing Fe concentration in the range of 0 to 160 mg Fe L^{-1} in soil solution. In fact, the Fe nutrient disorder is often concurrent with a deficiency of K and P in lowland rice. Sahrawat (2004) observed increased resistance to Fe toxicity when P and K were added to the soil.

One Fe uptake mechanism in rice is the contact between roots and Fe oxides described by Olsen and Brown (1980) showing that drops in redox potential (Eh) and pH lead to mobilization of Fe at the root-soil interface. The fraction that is directly exchangeable with the soil adsorption complex is called exchangeable Fe. During reduction and oxidation processes, the fractions that are potentially reducible are water soluble Fe, exchangeable and amorphous Fe, and crystalline Fe oxides. The reducibility of these various forms of Fe depends on their relative reactive surface area. A mechanism of defense of rice against toxic levels of Fe^{2+} and Al^{3+} has been proposed by Shamshuddin et al. (2013). First, plant roots create an oxygenated area around their immediate environment which can catalyze the precipitation of Fe hydroxides (Moorman and van Breemen, 1978). Secondly, plant roots release citric, oxalic and malic acid amounts proportional to Al excess. These acids, in turn, chelate the Al^{3+} and render it inactive, reducing its availability and toxicity. Therefore, sustainable practices should be promoted to alleviate the effects of toxic metals, create an environment favorable for rice survival and improve the quality marginalized agricultural land containing acid sulfate soils.

2.3 Potential management solutions

2.3.1 Rice varieties for iron toxicity in lowlands

One strategy for improved rice production is the use of rice varieties adapted to surviving under stresses such as hypoxia, acidity, and nutrient toxicity that are associated with lowland areas. The African variety, *Oryza glaberrima* Steud., is considered the most resistant to several biotic and abiotic stresses, including Fe toxicity. Sahrawat and Sika (2002) found a higher tolerance among *O. glaberrima* cultivars, which did not show any symptoms of Fe toxicity as did *O. sativa* cultivars. While management practices may be useful, these authors suggested the problem could be solved with appropriate field management and development of tolerant varieties.

Winslow et al. (1989) suggested that combining a resistant genotype with high ridging could reduce yield losses on Fe toxic soils. They observed a 245% increase in yield with the resistant genotype ITA247 of *Oryza sativa* L. planted on 120 mm wide ridges in a severely toxic paddy. Asch et al. (2005) suggested that a leaf bronzing score and tissue Fe concentrations should be used as parameters to discriminate resistant genotypes, but yield reductions from Fe toxicity can occur without any foliar symptoms (Abifarin, 1988). Moreover, Audebert and Sahrawat (2000) suggested that tolerance is based primarily on avoidance strategies (e.g., root oxidation of Fe^{2+} to Fe^{3+} decreasing transport from root to leaves) and tissue tolerance (e.g., photosynthetic potential sustained at high Fe concentration in the leaves). Overall, a better understanding of the physiological mechanism of Fe tolerance is an important piece of an improved plant breeding program.

2.3.2 Water management

Water management and field drainage practices aim to maintain the balance between reduction and oxidation conditions in the root zone that are responsible for the drastic shifts in soil chemistry that exacerbate Fe toxicity. Many Senegalese farmers have practical problems when attempting to improve drainage conditions and percolation in their paddies due to the limited elevation differences (Hanhart et al., 1997). Drainage and percolation rates are regulated by increasing the head between the field water level and the water level in the open drains or by decreasing the open drain distance (Hanhart et al., 1997). The strategy of improving drainage efficiency is based on the combined effects of reducing the exposure of pyrite to atmospheric oxygen and subsequent re-oxidation of sulfur compounds and ferrous Fe. Keeping the soil permanently water saturated suppresses sulfuric acid formation, which can also be neutralized through application of alkaline agents such as lime. An increase in soil pH does not always occur and sometimes, takes years to achieve (Creeper et al., 2012). Mathew et al. (2001) found that the acidity in the subsurface of the drained area was lower throughout the cropping period and the Fe transformations of those areas did not affect rice cultivation. Finally, water management has broader implications for ecosystem function in agricultural regions that should be considered. For example, Joukainen and Yli-Halla (2003) observed that drained soils may contribute significantly to the load of acidity in nearby rivers, particularly when sulfidic layers are close to the soil surface. Therefore, drainage needs to be adequately planned and combined with treatment of acidic discharge (White et al., 1997).

Given recent rainfall deficits and long-term water deficiency, controlled drainage practices must also plan for a reduced outflow and increased water use efficiency (Evans et al., 1995) for sustainable rice production. In this context, Shao et al. (2008) studied the use of controlled irrigation and drainage (CID) that combines controlled irrigation and alternate drying-rewetting practices. The goal of CID is to maintain an efficient depth of water for submergence while also capturing more drainage water for later use. Their results showed that CID at the tillering stage produced lower grain yield than CID applied at the booting, flowering or milky stages and the importance of flooded duration.

2.3.3 Soil amendments and advantages

2.3.3.1 Liming material

A variety of liming materials are used in agriculture to raise soil pH, but all function via the carbonate-bicarbonate equilibrium system to neutralize soil acidity (Adams, 1984). Their purity is typically expressed as the calcium carbonate equivalent (CCE), which can range from 50 to >105% for carbonate based liming materials. Unlike fertilizers that have application rates based on plant nutrient requirements, liming materials are applied in large amounts to change overall soil chemistry. Such liming agents can also impact desirable physical soil characteristics changes. For example, liming may improve soil structure and porosity which thereby decreases bulk density and enhances water retention. In fact, the higher concentration of Ca^{2+} and ionic strength promotes the flocculation of soil particles and therefore increases soil aggregation. Structural stability depends on the formation of the aggregates and activity of the native soil microorganisms (Chotte et al., 1998). Fageria and Baligar (2008) suggested the application rate for lime should depend on the type of soil, nutrient status, quality of the liming material, crop species, and cost. Lime requirement must be regularly monitored and lime applied periodically to neutralize acidity over the long-term.

The use of a liming agent in acid soils can improve soil properties and increase crop yield regardless of the type of crops (Tate, 2000; Lee et al., 2008). Beneficial effects include increased pH and adsorption/precipitation of toxic elements such as Al^{3+} and Fe^{2+}, which creates a better environment for microbial activity that mineralizes and increases availability of essential plant nutrients. This results from modifications in soil properties such as an increase in enzyme activities and changes in the microbial community. For example, Lee et al. (2008) observed that simply using oyster shells as a liming agent can increase available P and exchangeable cations that modify nutrient status and stimulate microbial activity, particularly enzyme activities such as urease and alkali phosphomonoesterase, which increase availability of soil nitrogen and phosphorous, respectively. These authors also highlighted the effect of soil texture on the efficiency of lime applied, as they found a higher liming effect on pH in sandy loam soil than in silt loams. Ai et al. (2015) showed that effectively raising soil pH with application of lime, fly ash, and chicken manure could increase rice yield. Improvement of soil physical parameters such as soil aggregate stability, macroporosity, and available water capacity can also be observed (Hati et al., 2008).

In the Casamance lowlands specifically, Beye (1972) compared calcium carbonate, calcium silicate and crushed oyster applied at rates of 3 Mg ha^{-1} and 6 Mg ha^{-1} to raise pH during rice growth. At harvest time, the amendments had achieved an increase of 0.5 pH unit for the first rate and 1 pH unit for the second rate. Furthermore, all amendments resulted in increased yield compared to the control except for 3 Mg ha^{-1} of crushed shell. These results confirm the importance of quantity and quality of liming material that is applied. In fact, the quality of the liming material depends on its neutralizing value, particle size distribution, and reactivity. Consideration of liming materials is particularly critical because of high costs in Senegal. A better understanding of their reactivity along with research on organic amendments that may have the same effects are needed for establishing sustainable options for reclaiming Fe-affected soils.

2.3.3.2 Biochar

Biochar is created by pyrolysis of plant material (wood and crop residues), which produces a stable product with high carbon content. Its water holding capacity and pH depend upon feedstock type and process of production, but biochar can contribute to soil fertility and yield increase (Lehmann et al., 2011; Manickam et al., 2015). The porosity of the biochar, which increases with the pyrolysis temperature, can facilitate colonization by microorganisms and help support increased microbial biomass and activity (Warnock et al. 2007) that could be beneficial for high yield. The effect of biochar on yield depends on rate applied and soil type (Manickam et al., 2015).

The properties of biochar suggest that it may also have great potential to remove contaminants and heavy metal cations from aqueous solution and reduce their mobility and availability. Its high surface area and predominantly negative charge (for most types of biochar) contribute to the high CEC found in soils that are rich in biochar (Liang et al., 2006). Porosity and surface area of biochar increases as the temperature of pyrolysis increases (Bird et al., 2011). Chen and Chen (2009) related the increased surface area of the biochar to the deprotonation of functional groups such as aliphatic alkyls and esters through high pyrolysis temperatures, thereby increasing its sorption capacity. As a result, biochar can serve as an adsorbent to remove chemical elements from the media, and, thus, reduce their availability. Dang et al. (2015) reported that *Eucalyptus* biochar and wheat biochar produced at 550°C and 450°C had higher retention of protons, Fe, and Al compared to compost, wheat straw, pea straw, wheat and poultry biochar produced at 550°C. The difference in removal ability or metal adsorption/desorption between the different organic materials was related to the organic-C composition, particularly the aromatic carbon structures such as aryl, O-aryl, O-alkyl, and ketone groups. Their results also suggest that the pH could influence the binding of metals to organic materials since metal binding increases as pH increase.

3. MATERIAL AND METHODS

3.1 Project location

Casamance is located in the southernmost part of Senegal and is divided into three ecological zones (the Lower, Middle and Upper Casamance) based on climate, ethnic composition, and primary crops. This research was conducted at an experimental field site (Plot "P9") at the Senegalese Research Institute for Agronomy in Djibelor (12°33'N, 16°19'W, 33 m altitude), located 5 km from Ziguinchor in lower Casamance.

The Lower Casamance (Figure 1) has a subtropical climate with a mean annual temperature of 31.5 °C. The average annual rainfall is 1,200 mm, which primarily falls between June and October. Although there is some inter-annual fluctuation, the peak rainfall remains centered in August and September. Precipitation amounts during the study period were recorded using a manual rain gauge at the Djibelor station. Dependent upon the timing of the precipitation, the growing season for rice cultivation in the area is typically from June to December. The lowland area is dominated by mangroves that are concentrated in the estuary of the Casamance River, which flows through the region. Tidal influence is important and leads to salt intrusion, which can be exacerbated by decreased rainfall that can cause variable local increases in salinity, depending on topography and location.

The soils that originate from mangrove habitats have a distinct pedological evolution that leads to Fe-toxicity (Prade et al., 1989-1990),. Prade et al. (1989-1990) described the presence (Figure 2) of fossilized materials of mangroves (zones A and B in Figure 2) in deep horizons, followed mainly by differentiated soils of ancient mangroves and potentially or actively acid sulfate soils (zones C and D) in the region. In this system, the soils located in the upper terraces of the topography are not saline (e.g., zones A and B), but on the lower terraces (zones C to E) soils need efficient salt leaching by shallow drainage at the beginning of the rainy season to allow flooded rice growth. These lower soils are described as potential acid sulfate soils and are typically subject to Fe toxicity. In Casamance, rice production occupies 116,000 ha and is practiced mainly in lowland areas that benefit from a good water supply. Agricultural operations are traditional, conducted with hand tools, and the use of chemical fertilizers or soil amendments is infrequent. Consequently, yields are low and are estimated at 3Mg ha^{-1} for lowlands rice and 1.5-3 Mg ha^{-1} for upland varieties.

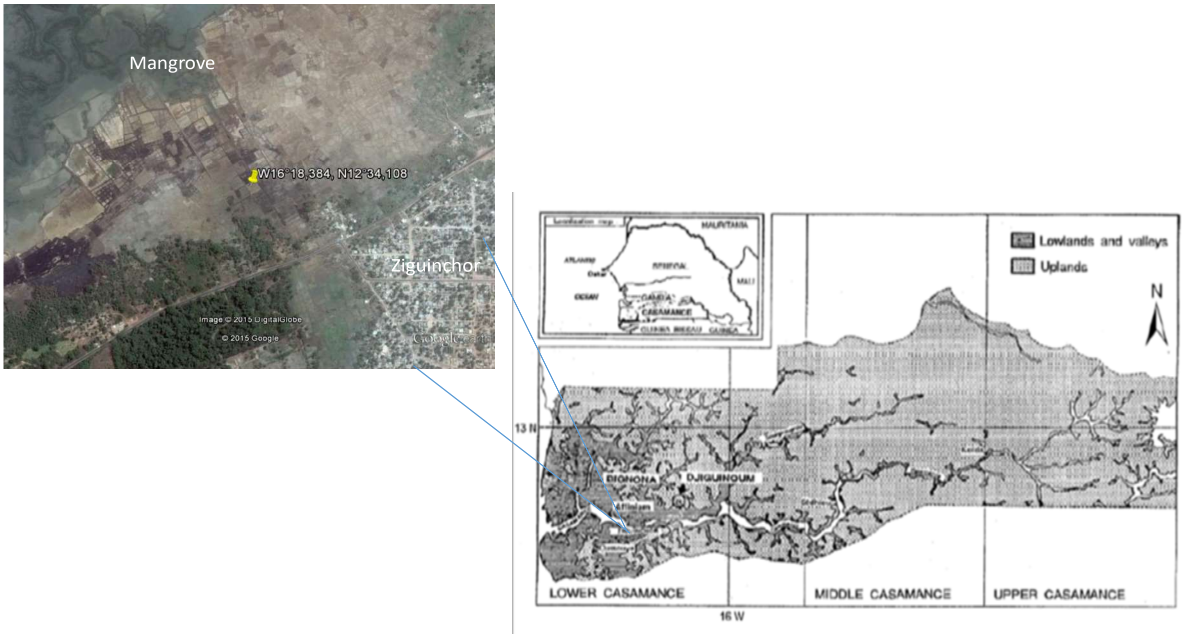


Figure 1. Map of the Casamance region of Senegal (Barry, 2009) and the location of the field experiment.

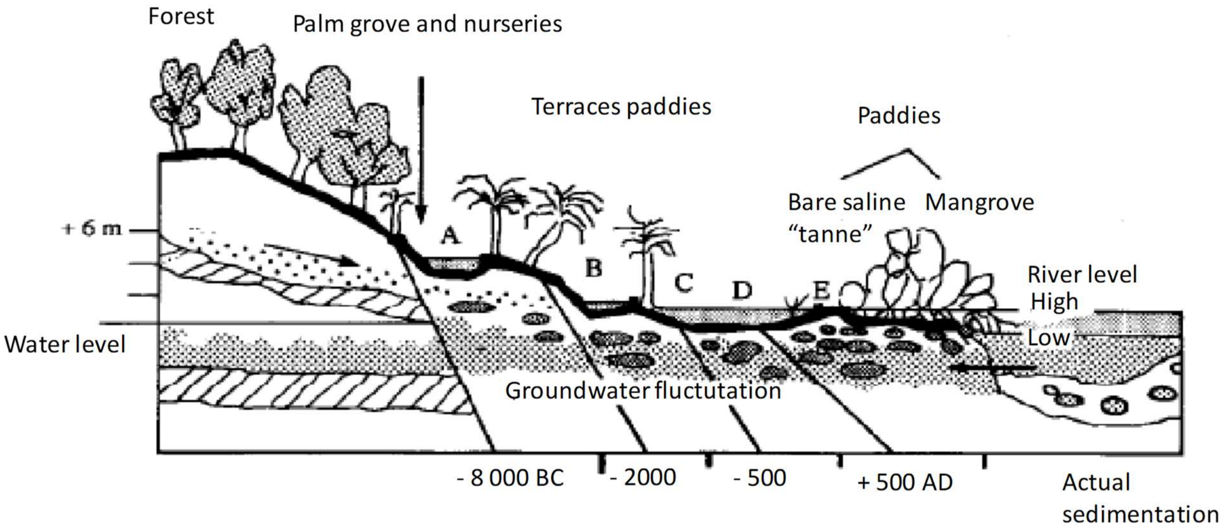


Figure 2. Localization of rice fields in Lower Casamance (Prade et al., 1989-1990). A and B: upper terraces, deep soils from colluvium (A) or alluvium (B), salt and sulfates at depth. Plots studied: Djibelor (E 6 to 8), Mahamouda, and Affiniam. C and D: lower terraces, potential and acid sulfate soil, variable salinity, often high. Plots studied: Djibelor (P4 and P25), Mampalago, Loudia ouoloff (12 to 18), karounate, Loudia ouoloff (7 to 11), Niambalang. E: saline marshes with partly containing bare surfaces "tannes" and mangroves. Plots studied: Loudia-Ouoloff (1 to 6) and Diakene-Diola). (*Figure used with IRD (ex ORSTOM) permission, Appendix A*).

3.2 Laboratory incubation

Prior to the field experiment, composite soil samples (0-10cm depth) from the proposed research plot area were collected and characterized. The soil type is typically sandy clay loam and acidic with a water pH of 3.3, with the following chemical properties determined by the Mehlich I method (as described in the analysis section). Cation exchange capacity is poor (10 $cmol_c\ kg^{-1}$) with plant available P and K concentrations estimated at 7 mg L^{-1} (moderate) and 14 mg L^{-1} (low), respectively. Fe, Mn and Mg concentrations were 119 mg L^{-1}, 9 mg L^{-1} and 207 mg L^{-1}, respectively. An initial incubation experiment was set up to test the capacity of the planned experimental amendments (biochar, powdered oyster shell, biochar + powdered oyster shell) to promote a rise of the pH, degradation of organic matter, and a decrease in Fe concentration in the local soils. A factorial complete randomized design was set up in triplicate. A subset of replicates comparing the shell+biochar amendment under a range of wet to saturated conditions was set up simultaneously to the other treatments.

The biochar, from red maple (*Acer rubrum* L.), contained 773 mg C g^{-1} and 1 mg N g^{-1}. The powdered oyster shell had a total neutralizing value ($CaCO_3$ equivalent) of 102%. The soil was pre-incubated at 60% water holding capacity (WHC) for one week. It was then adjusted to 80% of WHC and 30g of soil was mixed with each amendment at a rate of 1% or 15 Mg ha^{-1} rate in each 100 ml pot. The mixture was incubated at 28°C for two weeks, and sampled at 0, 2, 7, and 14 days. Soil pH was determined using a 1:1 soil:water ratio and CO_2 emissions were determined from gas samples analyzed on a gas chromatograph (GC-2014, Shimadzu, Japan). Iron concentrations were determined by shaking 2.5 g of soil with 25 ml of 0.05 M EDTA for 60 minutes in an incubator shaker at 110 rpm and 28 °C. The extracts were filtered through a 0.45 µm membrane and analyzed with inductively coupled plasma-atomic emission spectrometry (ICP-AES, Arcos FHS16, NJ, USA).

3.3 Field experiment

3.3.1 Hydrology: Monitoring well and Piezometer installation

A monitoring well and two piezometers were installed in one corner of the experimental paddy at different depths to monitor groundwater levels during the crop growth period (Figure 3). Depths were determined from the description of the soil profile and each one was installed in an individual borehole made with an auger. Each piezometer consisted of perforated PVC pipe opened at both ends, with the lower end located at the depth of interest (Cherry et al., 1983). The well (labelled "w" in Figure 3), was perforated from the ground surface to the bottom of the pipe and was installed to a depth of 2 m, which corresponded to the location of the local water table. The bentonite seal installed at the ground level prevented water flowing down along the pipe from the surface. The two piezometers (commonly called "nested piezometers") were perforated only at the bottom end and were located at deep ("dp", 1.42 m) and shallow depths ("sp", 0.29 m) to assess the vertical variations of the groundwater level. To construct these, sand was first filled in the borehole and then the piezometer was installed. Next, sand was placed along the screen (perforated zone), which was protected by a nylon filter cloth to avoid accumulation of silt and sand in the piezometer. A bentonite seal was then added to the top of the sand column to prevent water from flowing down along the pipe. Finally, the field soil was backfilled over the bentonite up to the surface level, where an additional bentonite seal was placed to further prohibit surface water entry. Both the well and piezometers were capped to avoid contamination and rainfall.

Figure 3. Well and piezometers installed near the experimental field plots in Djibelor, Senegal.

3.3.2 Characterization of soil amendment materials

Amendments were applied to achieve a target pH of 6.2 during field preparation, one month before transplantation of rice seedlings. Amendment treatments included biochar from *Eucalyptus camadulensis* sieved at 5 mm, oyster shell powder sieved at 1 mm, and agricultural lime. Biochar was produced by pyrolysis of eucalyptus wood at 600°C to 750°C for 28 h. In order to determine the lime requirements for the experiment, pH was measured in the field with a portable pH meter and in the laboratory and soil buffer pH determined. All measurements yielded a pH lower than 3.3, indicating a need for liming. Physical and chemical properties of the biochar and shell are described in Table 1.

Biochar pH and electrical conductivity (EC) values were determined from a biochar:water ratio of 1:20 after 1.5 hour agitation using a pH meter (ATTorion) and electrical conductivity meter (VWR international, Model 2052). Total P, Ca, Mg, K, and Na were quantified by acid digestion (USEPA, 1996; as described in analysis section) of 0.5 g biochar. Total elemental composition was evaluated by inductively coupled plasma-atomic emission spectrometry (ICP-AES) and available nitrate (NO_3^- -N) and ammonium (NH_4^+-N) were extracted with 2M KCl solution (Rayment and Higginson, 1992). CEC was determined using 1.0 g biochar and a sequential extraction was performed with successive solutions of 50 mL 1 N ammonium acetate at pH 7 followed by 90 mL 2 N KCl in two fractions (50 ml then 40 ml). Ammonium was quantified by flow injection analysis (Lachat, Hach Company, USA) and exchangeable cations were determined by ICP (Rajkovich et al., 2012). Total C and N were determined by dry combustion using a vario MAX CNS Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

The shell sample was analyzed by Waypoint Laboratories, Inc. (Richmond, VA, USA). The moisture content was determined by drying the shell material in the oven at 105°C (AOAC 1998) and the neutralizing value (%CCE) was estimated using the AOAC 955.01 method (AOAC, 1998). The sieving was performed using US standard sieve sizes and the Effective Neutralizing value was calculated from CCE, 8, 20, 40, 60, and 100 mesh sizes values.

Table 1. Chemical and physical characterization of the biochar and shell amendment materials used in field research plots.

Parameters	**Eucalyptus biochar**	**Oyster shell (pulverized)**
Moisture %	4.29	0.23
Ash %	67.68	-
pH water	7.6	-
Electrical Conductivity dS/m	0.12	-
C %	16.24	-
N %	0.16	-
C/N ratio	104.42	-
Effective Neutralizing value (%)	-	61.89
Total Neutralizing Value (%)	-	102
% passing 20 mesh, %	-	83.6
% passing 60 mesh, %	-	48.7
% passing 100 mesh, %	-	38.8
CEC meq/100g	44.32	-
Ca_plant available mg/Kg	133.67	-
K_plant available mg/Kg	4	-
Mg_plant available mg/Kg	8.94	-
Na_plant available mg/Kg	1.27	-
Total P mg/kg	1730	-
Total Ca mg/kg	9043	-
Total K mg/kg	9089	-
Total Mg mg/kg	2275	-
Total Na mg/kg	458	-

3.3.3 Experimental Design

The field experiment was conducted over two growing seasons (2014 and 2015) and was laid out in a 2 x 4 factorial split plot design with four replicates (Figure 4). The main plots consisted of two planting methods: flat or raised beds. The subplots consisted of four soil amendments: control, which received no amendment other than fertilizer (C); agricultural limestone (Li) at 13 Mg ha^{-1}; oyster shell powder (Sh) at 13 Mg ha^{-1}; and biochar (Bi) at 15 Mg ha^{-1}. Each plot measured 4 m x 3 m and plots were separated by 1 m buffers; blocks were separated by 2 m. Fertilizers in the form of NPK 15-15-15 (200 kg ha^{-1}) and NK 13-0-50 (180 kg ha^{-1}) were applied equally to all treatments, including the control plots, at transplanting time. An additional 150 kg N ha^{-1}of urea was applied as 75 kg N ha^{-1}at the time of tiller onset (21 days after transplanting) and again at panicle initiation (45 days after transplanting). In the second year of the experiment, plots received only fertilizers without any additional soil treatment amendments.

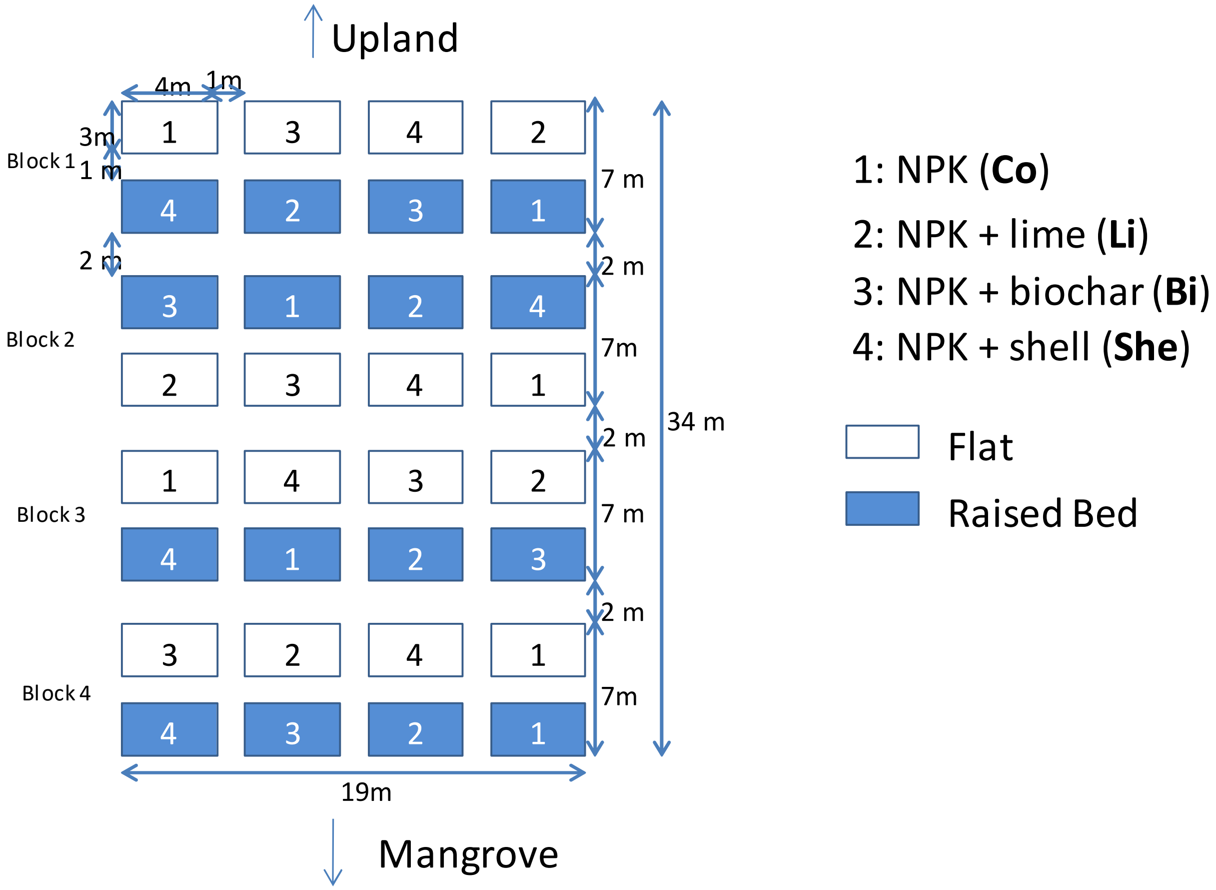


Figure 4. Experimental design and field orientation of experimental blocks, treatments, and replicate plots in Djibelor, Senegal.

3.3.4 Field preparation and crop management

The field was ploughed manually before setting up the experiment. Raised beds were formed with a local tool, the "Kayendu", with a top of the bed 1 m wide x 20 cm high and a furrow width of 40cm. Each replicate plot was 4 m x 3 m including 3 ridges (1 m x 3 m) in the bed system. The flat system consisted of 4 m x 3 m plots with unraised 1 m x 3 m planting areas. Clods were broken in bedded and flat plots to provide favorable tilth conditions for easy transplanting of seedlings.

The rice variety used in this study was "BW248-1", which has a potential yield of 6 t ha^{-1}. Following traditional practice for Casamance, the rice was sown at the nursery (in upland soils) in a raised bed at a spacing of 20 x 20cm with a fertilization rate of 40 kg ha^{-1} of NPK 15-15-15. Three week-old seedlings were transplanted to the field as two seedlings per hill at a spacing of 20 x 20 cm. Weeding was done by hand 21 d and 45 d after transplanting. Water management was limited to maintaining submergence throughout the crop growth period except during tiller onset to avoid inhibition. The water level was also decreased for fertilizer application and after submergence due to heavy rains. At plant maturity, water was drained from the field.

3.3.5 Field sampling and analysis

Hydrology

The water level was measured weekly from the well and piezometers and water samples were collected in 60 ml bottles at the same time. pH and electrical conductivity were determined using a portable pH/conductivity meter (Hanna Instruments, Inc, USA).

Yield parameters

At maturity, the crop was harvested from a designated area of 1.6 m x 1 m in each plot. Plant height and yield components including effective tillers $hill^{-1}$, number tillers m^{-2}, number panicles m^{-2}, and mass of 1000 grains were recorded from ten randomly selected hills in each subplot. The grain yield (t ha^{-1}) was determined for the entire harvested area avoiding the border rows.

Soil sampling

The site soil profile was described prior to the experiment. Samples were taken from each horizon, identified, and analyzed. Particle-size analysis was performed by the pipette method (Gee and Bauder, 1986) and organic matter percentage (modified Walkey-Black method), pH in water (soil:solution ratio of 1:1), and acid extractable cations (Mehlich 1) were determined by the Virginia Tech- Virginia Cooperative Extension Soil Testing Laboratory (Maguire and Heckendorn, 2011). Total Fe was evaluated by acid digestion (EPA 3052) and soil acidity, potentially generated by sulfur oxides, was determined by a modified H_2O_2 oxidation technique followed by whole soil titration (Perxoxide Potential Acidity - PPA)

(Orndorff and Daniels, 2004). Total sulfur was measured with a Vario Max Elemental analyzer (Elementar Analysensyteme, Hanau, Germany) after combustion at 1150°C.

During the experiment, a composite sample was collected from the 0-10 cm depth of each subplot with an auger at four different times during the growing season: transplantation, panicle initiation, flowering, and harvest. Each sample was divided into three aliquots: one was stored at -20 °C for biological analysis, one was sieved, air dried and sent to Virginia Tech for chemical analysis, and the third aliquot was sieved, air dried, and stored at Djibelor station for archiving.

pH

All samples were analyzed for pH in a soil:water ratio of 1:1f. The mixture was stirred and allowed to stand for 10 minutes before measuring on a pH meter (ATTorion). The meter was calibrated with standards (pH 4, pH 7, and pH 10).

Electrical Conductivity

At the beginning of each growing season, the saturated soil electrical conductivity (EC) was measured at transplanting time to quantify soil salinity. In a 100 ml beaker, 90 mL of soil was added with enough deionized water to saturate the sample. The slurry was stirred and then left to settle for 2 hours before filtering through a Whatman nº2 42 mm filter paper (Whatman International Ltd, England). Salinity readings were then performed on the filtrate with an EC meter (VWR international, Model 2052).

Mehlich 1 (Dilute double acid) extraction

The Mehlich 1 (M1) extractant, also referred to as 'dilute double acid' solution, was used to assess the plant availability and extractable fraction of plant nutrients in the soil profile and experimental samples (Maguire and Heckendorn, 2011). This method is suited for soils with acid to neutral pH and low CEC (<10 meq100 g^{-1}). The soil samples (5 g) were reacted with 20 ml of an extracting reagent composed of 0.05 N HCl and 0.025 N H_2SO_4. The samples were shaken on a reciprocal shaker for 5 minutes at 180 oscillations min^{-1} and filtered through Whatman No.2 filter paper.

Exchangeable aluminum extraction

Exchangeable aluminum (Al^{3+}) was extracted with 1M KCl from 5g of soil for soil profile and experimental samples. The mixture was shaken for 1h on a reciprocal shaker. After extraction, suspensions were centrifuged for 30 min at 1700 g and filtered through a 0.45 µm Whatman membrane filter. Al concentrations in the supernatants were determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Arcos FHS16, NJ, USA).

Particulate organic matter

The particulate soil fraction represents the >50 µm size that is separated by wet sieving (Cambardella and Elliott, 1992). The particulate organic matter (POM) fraction is part of the labile (easily decomposable) pool of soil organic matter (SOM) and was quantified in samples from 2014 only. For each sample, 1 g was dried at 105°C for air-to-oven dry mass corrections and 20 g of dry soil were collected in pre-weighed 175 ml Nalgene bottles for fractionation. Next, 60 ml of 5% sodium hexametaphosphate solution (50 g L^{-1}) was added and samples were shaken for 18 hours on a reciprocal shaker on high speed. After shaking, the mixture was poured through a 53 µM sieve to recover the supernatant. The samples were rinsed with deionized water until clear and the resulting POM (i.e > 53 µm) fraction was dried in a pre-weighed tray at 105°C. Dried materials were milled and transferred to scintillation vials. The remaining fraction was freeze-dried, hand-ground with a pestle and mortar, and then transferred to scintillation vials. All samples were dried at 105°C overnight then an aliquot weighed into tin cups for C and N analysis by the CE Elantech 1112 elemental analyzer.

Iron sequential extraction procedure

A sequential extraction procedure (SEP) for Fe was conducted following a protocol published by Claff et al. (2010) with some modifications. This method allows the assessment and separation of Fe into six geochemical fractions. Briefly, the extracting ratio of 1:20 (soil:solution) was applied to a 1 g soil sample. The first step in the procedure was to extract readily soluble Fe and exchangeable Fe with 1M magnesium chloride ($MgCl_2$) for 1h. Second, carbonates and poorly ordered sulfides and oxides were extracted with 1 M hydrochloric acid (HCl) for 4h. The third extraction used 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) adjusted to pH 10.4 for 16h to target metals bound to organic matter. Fourth, the crystalline Fe oxide mineral fraction was extracted with 0.35 M acetic acid/0.2 M sodium citrate buffered with 50 g L^{-1} sodium dithionite (CBD) for 4h. Fifth, the pyrite fraction was extracted using concentrated nitric acid (HNO_3). Finally, the residual fraction of metals bound to the soil matrix was extracted using the hot digest process (aqua regia 3:1, HNO_3/HCL) in a water bath at 96°C. Reactions were performed at room temperature unless otherwise specified, in constantly agitated (110 rpm) acid-washed 50 ml centrifuge vials and for the specified time. At each extraction step, the supernatant was centrifuged for 10 min at 4000 rpm, and then filtered through 0.45 µm syringe filters. Extracts of steps 5 and 6 were separated by centrifugation only. The extracts were acidified with nitric acid to pH < 2 and solid phases were washed with 10ml of deionized water between extractions. The extracts were analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Arcos FHS16, NJ, USA) to calculate the percentage of the Fe mineral that was dissolved in each extraction step.

Total Fe

Total Fe in the soil profile and experimental samples was determined using EPA Method 3052 (USEPA, 1996). The digestion of 1 g of oven dried soil was performed with 12 ml of aqua regia (9 ml HNO_3 + 3 ml HCl) to predigest the sample. Next the vessels were sealed and heated for 15 minutes at 160°C (Maximum microwave power at 100%, 1600W) using the MARS Xpress microwave (CEM Corporation, Matthews, North Carolina 28106, USA). Fe concentration was then determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Arcos FHS16, NJ, USA).

Bacterial populations

The abundances of total bacteria and Fe reducing bacteria (IRB) were quantified using quantitative polymerase chain reaction (qPCR) during the 2014 growing season. Soil DNA extractions were performed on 0.25 g soil using the Mo Bio PowerSoil DNA Isolation-Kit (MOBIO Laboratories, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Nucleic acids were eluted in 50 µl of nuclease free water and stored at -20 °C for further analysis. DNA concentrations were measured and 260/280 ratios were determined using a NanoDrop fluorospectrophotometer (NanoDrop Technologies, Delaware, USA). Quantitative PCR was performed in an Mastercycler ep Gradient S thermal cycler (Eppendorf, USA) by reacting 1.25 µl each primer (10 µM), 12.5 µl iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc, USA), 5 µl template DNA (20 ng), and enough nuclease free water for 25 µl final volume. A qPCR amplification of the 16S rRNA for total bacteria was performed using the universal primers EUB338f (5'-ACT CCT ACG GGA GGC AGC AG-3') and 518r (5'-ATT ACC GCG GCT GCT GG-3') (Fierer et al., 2005). Reactions were initially incubated at 96°C (3 min) to denature all the DNA, followed by 40 cycles at 94°C for template denaturation (30 s), 55 for primer annealing (30 s), and 72 for extension (30 s). Reactions finished with an extra 72 for final extension (5 min). qPCR targeting the 16S rRNA genes of *Geobacteraceae* spp. (Fe reducing bacteria) was performed using the primers Geo564f (5'-AAG CGT TGT TCG GAW TTA T-3') and Geo840r (5'-GGC ACT GCA GGG GTC AAT A-3') (Cummings et al., 2003). Reactions were initially incubated at 94°C (4 min), followed by 35 cycles of 94°C (30 s), annealing at a 20 step touchdown program from 65° to 55°C in 0.5° increments (30 s), and extension at 72°C (3 min). Reactions finished with an extra 72°C step for final extension (5 min). Abundances and ratios of *Geobacteria* and total bacteria were calculated by comparing Ct values of unknown samples to a standard curve consisting of tenfold serial dilutions from 10^8 to 10 copies of a plasmid containing the target sequences.

3.4 Statistical analysis

Data were analyzed by multifactorial analysis of variance using JMP Pro 12 (SAS Institute Inc, USA). Repeated measurement analyses of variance (ANOVA) with the factors as amendments (four levels: control, biochar, lime, shell), planting method (two levels: flat and bed) and sampling time (two dates: transplanting and harvest) were conducted. Additionally, two-way ANOVA was performed to test the treatment effects on yield parameters at harvest. Normal distributions of the residuals were assessed using histograms and normal quantile plots. If the requirement was not met, data were arcsin transformed prior to analysis. Fisher's LSD tests was used to test differences among the means and determine statistical significance at $p < 0.05$. Principal components analysis (PCA), a multivariate analytical and visualization tool, was performed using JMP Pro 12 (SAS Institute Inc, USA) on soil chemical characteristics to explain variance in the data while reducing the number of variables to a few primary components. This method allowed identification of a group of variables or individuals and the relationships among the observed variables. Linear regression analyses with JMP Pro 12 were used to relate pH, extractable Fe, and nutrients.

4. RESULTS

4.1 Laboratory incubation

Adding soil amendments is expected to stimulate microbial activities that affect soil fertility, but the outcome of these processes depends on biotic and abiotic interactions in the soil environment. Therefore, the laboratory incubation was performed prior to the field experiment to evaluate the short-term capacity of the planned amendments (biochar and shell) to promote an increase in pH, increase degradation of organic matter, and decrease Fe concentrations, which are all key mitigation goals of Fe-affected soils specifically and acid sulfate soils more generally.

Over the course of the incubation, the shell and shell+biochar inputs significantly increased the pH ($p<0.05$) from 3.8 to 5.95 and 5.16 respectively (Figure 5). There was an interaction of measurement time and amendment (Table 2). There was no difference between the biochar and control treatments. The increased pH was likely due to the presence of carbonates in the shell material that neutralized the soil acidity. In addition, pH, CO_2 released, and Fe concentrations were all significantly ($p<0.05$) affected by the interaction of Time*Amendment during the laboratory incubation period, indicating that the treatment differences were increasing over time (Figure 5).

Table 2. Summary of two-way ANOVA results of the influence of amendments on pH, CO_2 released, and Fe levels during laboratory incubation ($n=3$, $p<0.05$).

Source of variation	df	pH	CO_2	Fe_{EDTA}
Amendment	3	<.0001*	<.0001*	<.0001*
Time	3	0.0024*	<.0001*	ns
Amendment*Time	9	0.0059*	<.0001*	0.0032*

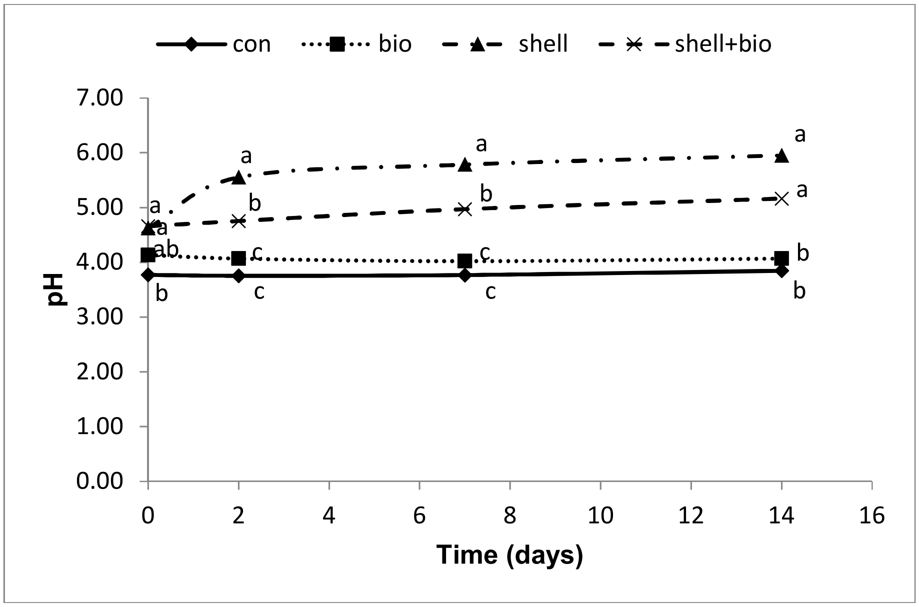


Figure 5. Soil pH (n=3) as affected by the different amendments during the incubation. Different letters denote significant differences by date during incubation.

C mineralization

Carbon mineralization is a biological process involving the breakdown of organic substances by microorganisms with the end products being CO_2, H_2O, and mineral nutrients. Relatively non-palatable C remains in the soil as humus. This process is often used to estimate soil functionality or fertility (Mutuo et al., 2006). In the laboratory incubation, the two amendments containing shell material resulted in a significantly ($p<0.05$) increased rates of CO_2 release compared to the control and biochar treatments (Table 3). In the shell amended plots, the respiration rates varied from 687 µg C h^{-1} g^{-1} soil on the first day to 116 µg C-CO_2 h^{-1} g^{-1} soil at the end of the incubation. In contrast, respiration rates in the biochar amendment alone varied from 164 µg C h^{-1} g^{-1} soil on the first day to 92 µg C-CO_2 h^{-1} g^{-1} soil at the end of the incubation and were not significantly different from the control. This can be explained by the intrinsic stability of the biochar C related to its condensed aromatic structure (Zhang et al., 2012).

Table 3. Rates of CO_2 efflux from soil amended during the incubation. Different letters denote significant differences between treatments by date ($p<0.05$).

	C mineralization (µg C-CO_2/h/g soil)			
Treatment	day 1	day 2	day 7	day 14
control	57 c	110 c	113 b	97 b
biochar	164 c	141 c	114 b	92 b
shell	687 a	298 a	150 a	116 a
shell+bio	378 b	227 b	139 a	130 a

Iron extraction was performed with EDTA and showed that soil amended with shell material contained the lowest amounts of extractable Fe (Figure 6), which varied from 19.77 to 24.14 mg Fe l^{-1}. In contrast, extractable concentrations were higher in the control and biochar treatments, indicating that there was increased availability of Fe. Overall, the biochar amended soils contained the highest extractable Fe concentrations at the end of the incubation. An increase of Fe concentrations over the time is observed for the control, biochar, and shell treatments Shell + Biochar treatment recorded a decrease in Fe concentrations during all sampling time of the incubation compared to initial time. When extractable Fe concentrations were measured in a subset of pots amended with shell plus biochar and incubated under both wet and flooded conditions, Fe availability increased significantly ($p<0.05$) by about three-fold under flooded conditions (Figure 7).

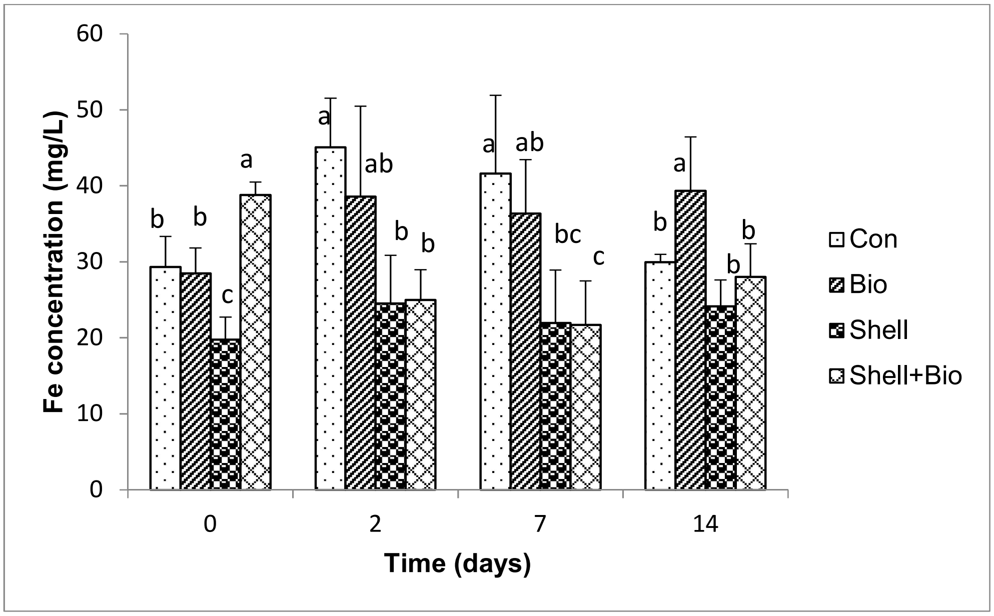


Figure 6. Soil extractable Fe concentrations (n=3) as affected by the different treatments during laboratory incubation. Treatments with different letters by date are significantly different (p<0.05).

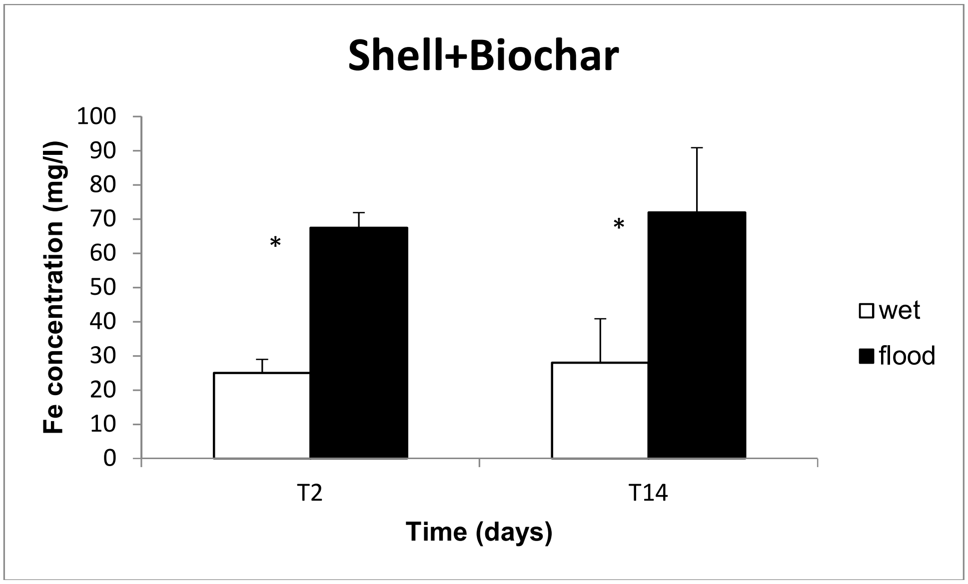


Figure 7. Comparison of extractable Fe concentrations (mg L^{-1}) from the shell+biochar treatment in wet and flooded conditions during lab incubation time.

4.2 Field experiment: Site Characteristics

Rainfall

The total rainfall recorded during the 2014 and 2015 growing seasons were 1390 mm, and 1435 mm respectively (Figure 8). Like most tropical Savannah, the precipitation was unevenly distributed, with the peaks centered in August and September (Figure 9). Recent years have experienced rainfall deficits with a transient drought, a late start, or an early cessation, all of which may impact the rice growth cycle and yield. However, during this experiment rainfall levels were close to the mean annual rainfall, suggesting that total rainfall was not a major factor in rice yield during this study.

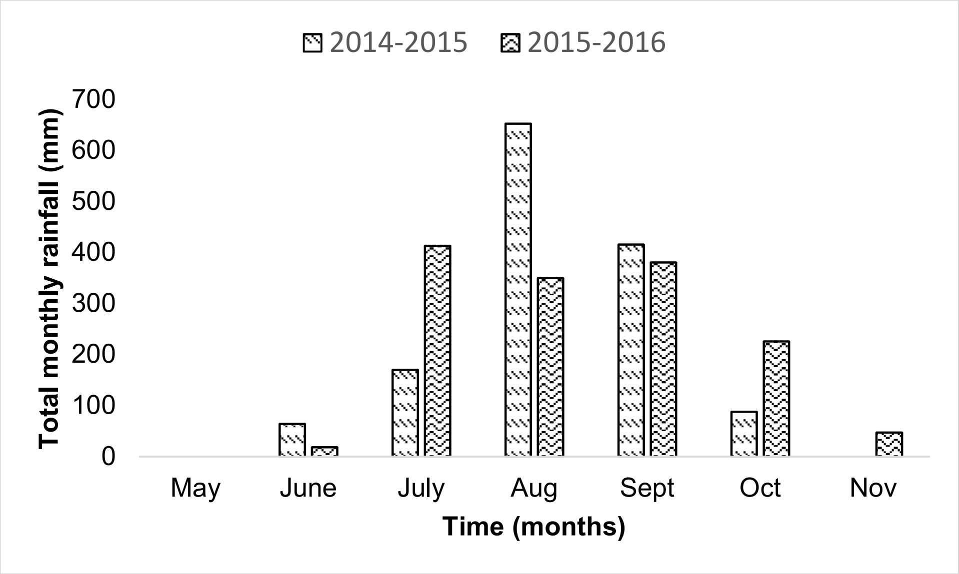


Figure 8. Amount of rainfall recorded during the two growing seasons (2014 and 2015).

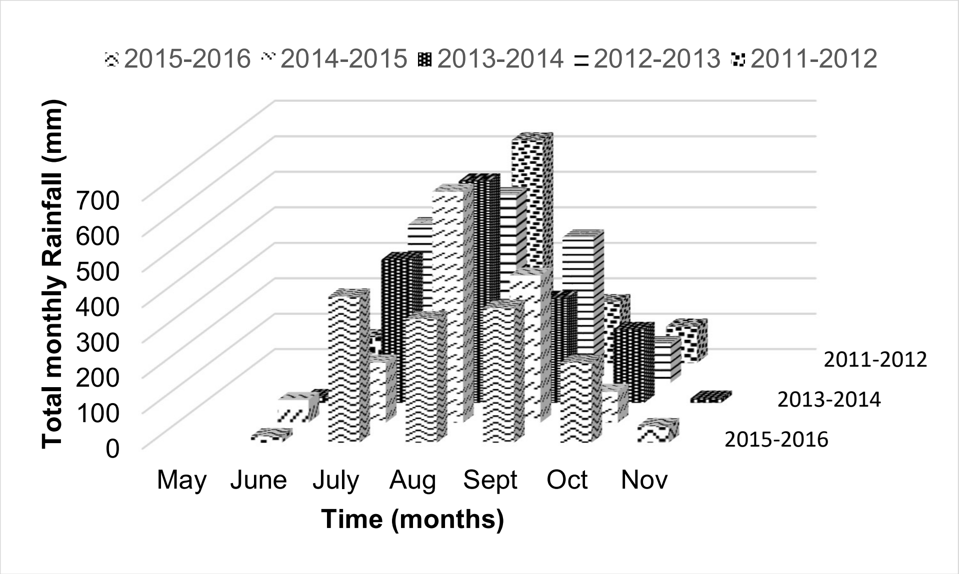


Figure 9. Monthly distributions of rainfall recorded during the last five years at the Djibelor research station.

Hydrology

During the rice growing season in 2014, the water table rose above the soil surface on August 16 in the experimental plot (Figure 10). Rice seedlings were transplanted on August 17 and the piezometers recorded a water head above 15 cm during the entire growing season. The electrical conductivity measured in the piezometers remained below 1.5 dS m^{-1}

during the growing season and the pH increased during the early season to peak at 7 in September before decreasing for the remainder of the season (figure 10).

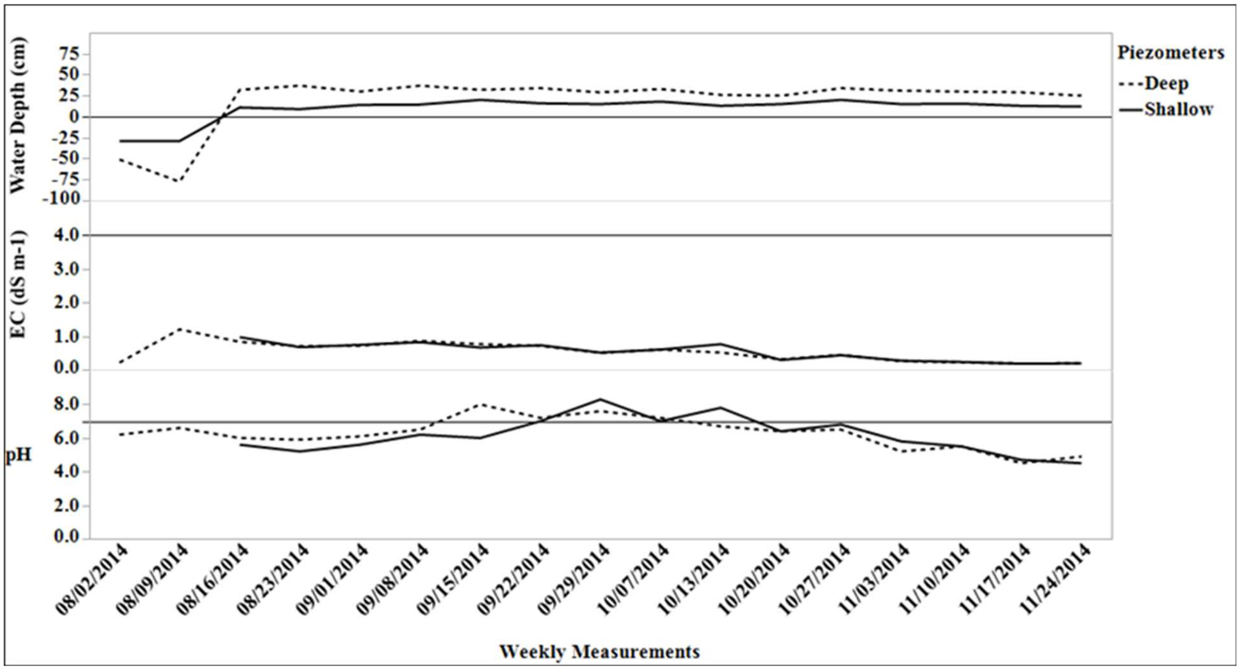


Figure 10. Weekly measurement of water table depth (top) in the two shallow and deep piezometers. Values for EC (middle) and pH (bottom) measured in water samples are plotted for the same times from measurements made on water samples for the 2014 growing season.

Description of the soil profile

The soil profile had two Apz horizons comprised of dark gray to grayish brown fine sandy loam that varied from subangular blocky to platy structure (Table 4). The lower Ap horizon was an older deeply plowed horizon while the upper Ap horizon was plowed on an annual basis. These plowed layers overlaid a sandy clay loam BA horizon with subangular blocky structure and dark gray matrix color resulting from saturation (figure 11). In the subsoil there were Btg and BCtg horizons dominated by gray to grayish brown sandy clay loams with subangular blocky structure. In the substratum, there were structureless massive brown and dark yellowish brown C horizons of loamy fine sand. The water table was at the base of the profile during the dry season. The soil is a member of the fine-loamy, kaolinitic, subactive, isohyperthermic family of Plinthic Kanhaplaquults. If a subgroup of Sulfic existed as defined for other great groups, it could be added to the Plinthic subgroup name to be more descriptive of the major properties. The Anthraquic subgroup could also be applied because of the anthropogenic flooding to grow rice. The parent materials are unconsolidated coastal plains sediments.

There is a major shift in soil particle-size at 58 cm depth, and a major shift in chemical properties at 110 cm depth. This soil has physical and chemical problems associated with elevated soluble salts and sulfur (Appendix B). The pH is low (≤ 3.5) in all horizons, particularly in the lowest two where it drops to 2.45 due to the oxidation of sulfides. Electrical conductivity is high in the Apz1 (56.75 dS m^{-1}) and the two C horizons (12.85 and 9.35 dS m^{-1} respectively). The elevated electrical conductivity at the surface is due to salt concentration following evaporation, while the high values in the Cjz horizon are due to influx of saline groundwater. There is a sulfuric horizon below 110 cm and a kandic horizon from 43 to 110 cm. Plinthite forms a discontinuous layer below 132 cm. Redoximorphic features were noted and formed as a result of extended saturation and reduction of Mn, Fe, and S during the rainy season, followed by oxidation and precipitation during the dry season. The yellowish redox concentrations are likely a form of Fe-sulfate minerals, most likely jarosite. The soil is hydric and has at least 15 cm of thickness near the surface with a depleted matrix of 60% or more with chroma 1 (BAg horizon). Therefore, it meets the properties of an indicator F3 (depleted matrix) as stipulated by the USDA-NRCS hydric soil indicators of the United States (USDA-NRCS, 2010).

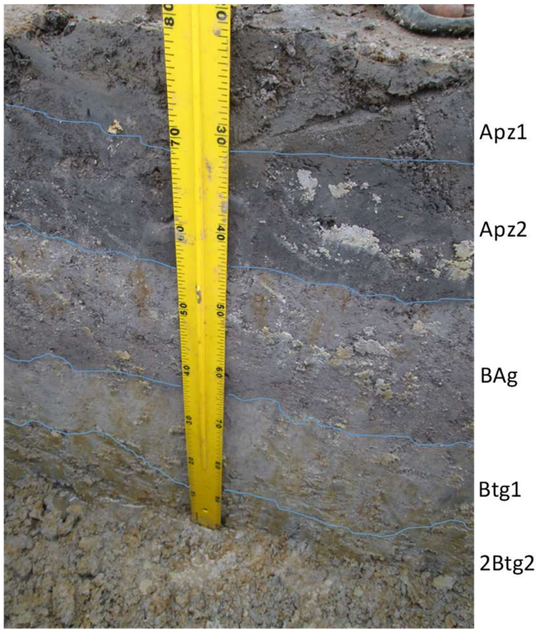


Figure 11. A representative soil profile of the Djibelor experiment research station (this picture was taken on January 4, 2016). The soil profile was described on July 8, 2014 before rice transplanting while the groundwater was still low.

Table 4. Physical, chemical and morphological characteristics of the soil profile described at Djibelor by Dr. John Galbraith and Ndeye Diallo.

Horizon name	**Soil description**
Apz1	0 to 13 cm; 10YR 2/2 fine sandy loam; weak, fine, subangular blocky structure; very friable; 5% 5YR 4/4 Fe pore linings and soft masses; extremely acid (pH 3.36); abrupt smooth boundary.
Apz2	13 to 22 cm; 10YR 3/1 sandy loam; weak, thick, platy structure; friable; % 5YR 4/6 Fe pore linings and soft masses; extremely acid (pH 3.36); abrupt smooth boundary.
BAg	22 to 43 cm; 10YR 4/1 sandy clay loam; moderate, medium, subangular blocky structure; friable; 10% 5YR 5/6 soft Fe masses and pore linings; extremely acid (pH 3.58); abrupt smooth boundary.
Btg1	43 to 58 cm; 10YR 5/1 sandy clay loam; moderate, medium, subangular blocky structure; friable; few faint clay films lining pores and ped surfaces;15% 5YR 4/4 soft Fe masses and pore linings; extremely acid (pH 3.29); gradual smooth boundary.
2Btg2	58 to 72 cm; 10YR 6/2 sandy clay loam; moderate, medium, subangular blocky structure; friable; few faint clay films lining pores and ped surfaces; 30% 5YR 4/4 and 7.5YR 4/6 and 10YR 6/6 soft Fe masses; extremely acid (pH 2.91); gradual smooth boundary.
2Btg3	72 to 90 cm; 10YR 5/1 sandy clay loam; moderate, medium, subangular blocky structure; firm; few faint clay films lining pores and ped surfaces; 40% 7.5YR 4/6 and 10YR 6/6 soft Fe masses; 20% N 4/0 depletion zones; extremely acid (pH 2.72); gradual smooth boundary.
2BCtg	90 to 110 cm; 10YR 4/2 sandy clay loam; moderate, medium, subangular blocky structure; friable; few faint clay films lining pores and ped surfaces; 40% 7.5YR 4/4 soft Fe masses; 20% N 4/0 depletion zones; extremely acid (pH 2.62); gradual smooth boundary.
2Cjz	110 to 132 cm; 10YR 4/3 loamy fine sand; structureless, massive; loose; 20% 7.5YR 4/6 soft Fe masses; 10% N 5/0 depletion zones; 2% 2.5YR 3/6 plinthite; extremely acid (pH 2.50); clear smooth boundary.
2Cjvz	132 to 150 cm; 10YR 4/4 loamy fine sand; structureless, massive; very friable; 35% 2.5YR 3/6 soft Fe masses; 5% N 2.5/0 Fe-Mn concretions; 15% N 5/0 depletion zones; 35% 2.5YR 3/6 plinthite; extremely acid (ph 2.45).

4.3 Field experiment: effect of incorporating amendments on soil properties

4.3.1 Electrical conductivity

Given that high concentrations of soluble salts and high electrical conductivity were observed in the upper horizon (0-13cm) of the soil profile (Appendix A), electrical conductivity was measured at transplanting time for each growing season. Concentrations were low to moderately saline in the soil (Table 5), suggesting that the drainage practices were sufficient to leach excessive salts from the soil, reduce salinity and allow rice growth.

Table 5. Electrical conductivity expressed in dS m^{-1} (mean ± std error; n=4) at transplanting time for each growing season (2014 and 2015).

Treatment	Electrical Conductivity (dS/m) 2014	Electrical Conductivity (dS/m) 2015
Control Flat (Cof)	3.95 ± 0.71	4.84 ± 0.1
Control Bed (Cob	3.18 ± 0.24	4.77 ± 0.1
Lime Flat (Lif)	5.40 ± 1.52	6.17 ± 0.84
Lime Bed (Lib)	3.81 ± 0.34	6.01 ± 1.05
Biochar Flat (Biof)	6.33 ± 1.64	4.80 ± 1.32
Biochar Bed (Biob)	3.63 ± 0.37	4.27 ± 0.84
Shell Flat (Shef)	8.04 ± 1.83	6.40 ± 1.84
Shell Bed (Sheb)	3.97 ± 0.39	3.36 ± 0.79

4.3.2 Principal component analysis of chemical parameters

Measured soil chemical characteristics included pH, extractable ions (P, K, Ca, Zn, Mn, Mg, and Fe), cation exchange capacity (CEC), base saturation (BS) and soluble salt (SS). Given that multiple soil characteristics were measured on soil samples in different treatments, principal component analysis (Boruvka et al., 2005) was used to analyze which sampling times showed the strongest treatment effects on overall soil chemistry during the 2014 growing season (Figures 12 and 13). This approach allowed us to account for multiple response variables simultaneously and better reflect the overall potential influences on the soil-plant system.

Among all of the principal component analyses (Figures 12 and 13), the first two components combined explained 69 – 74% of the total variation. Two of the sampling times, transplanting and harvest, displayed the strongest discrimination of treatment effects, so those two sampling times were chosen for subsequent statistical tests. At transplant time, pH, BS, CEC, Ca, were the primary factors that defined the first component (more than 80%) and the separation of the treatments. Specifically, the highest values for these factors were measured in plots amended with lime and shell. High positive loadings of pH, BS, CEC, Ca on component 1 corresponded to highest values or contents for those variables and high negative loadings of Fe, Mn, and Zn on component 1 corresponded to the lowest values for those variables. So, the PCA displayed a separation of samples along the first component PC1 where samples from shell and lime amended plots were distinct from samples collected from the biochar and control plots. This pattern was less clear at panicle initiation and flowering time. At harvest, the separation was again more evident but less distinct than transplanting. There was no clear distinction of points from raised or flat beds, suggesting that this practice did not have significant effects on overall soil chemistry. Also the ordination of treatments according to component 1 and 2 from PCA analysis showed that the variables taken together can differentiate treatments among them but it does not edify on the significance of treatment effects on each variable that needs to be determined.

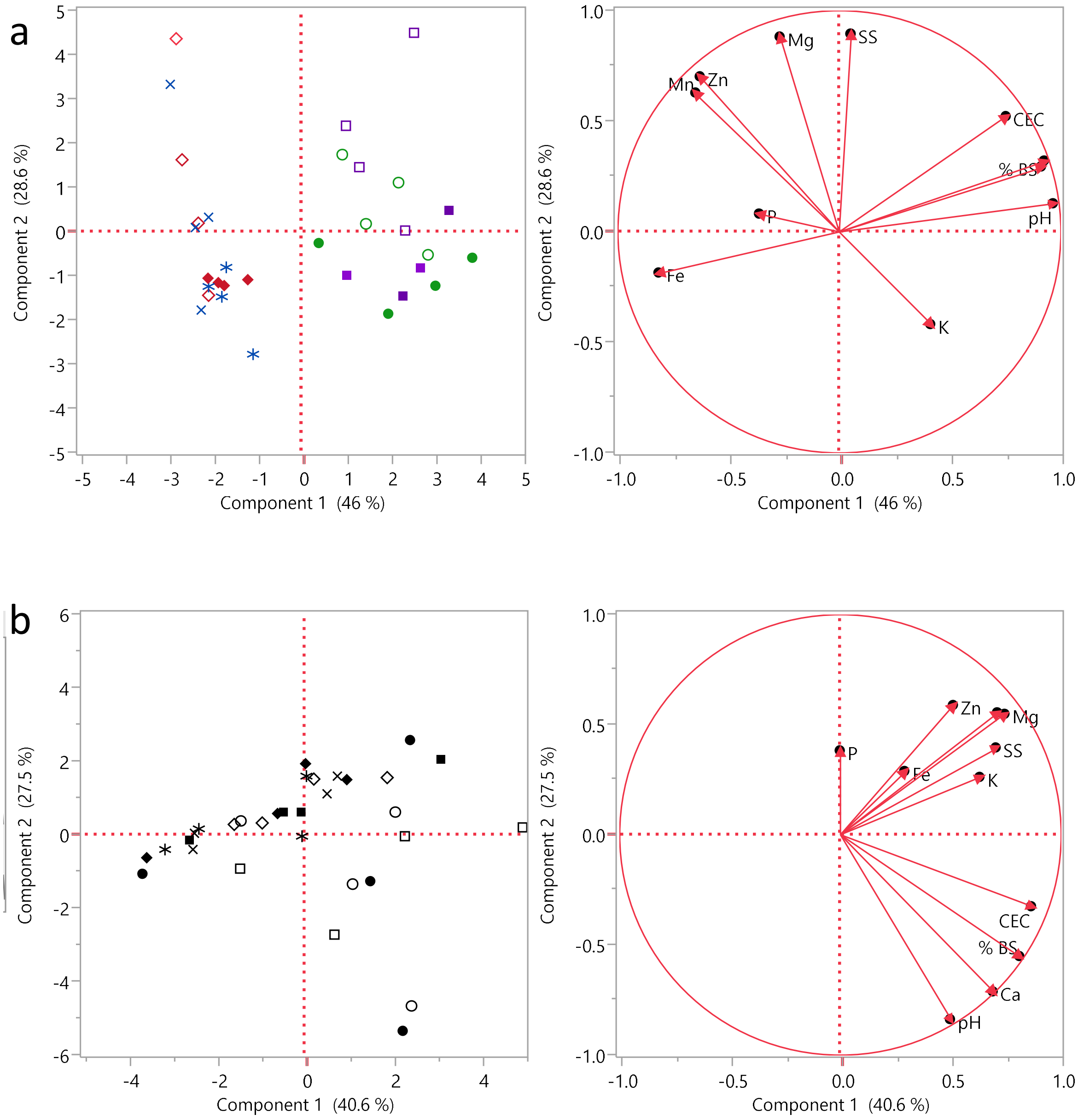


Figure 12. Principal component analyses (PCA) of soil chemical characteristics from the experimental plots at transplanting (a) and panicle initiation (b) times. The first and second principle components are plotted and the percentage of variance explained by each component is indicated within parenthesis on each axis. Closed symbols are raised bed treatments and open symbols are flat treatments. ◊: biochar; □: Shell; ○: Lime; x: Control.

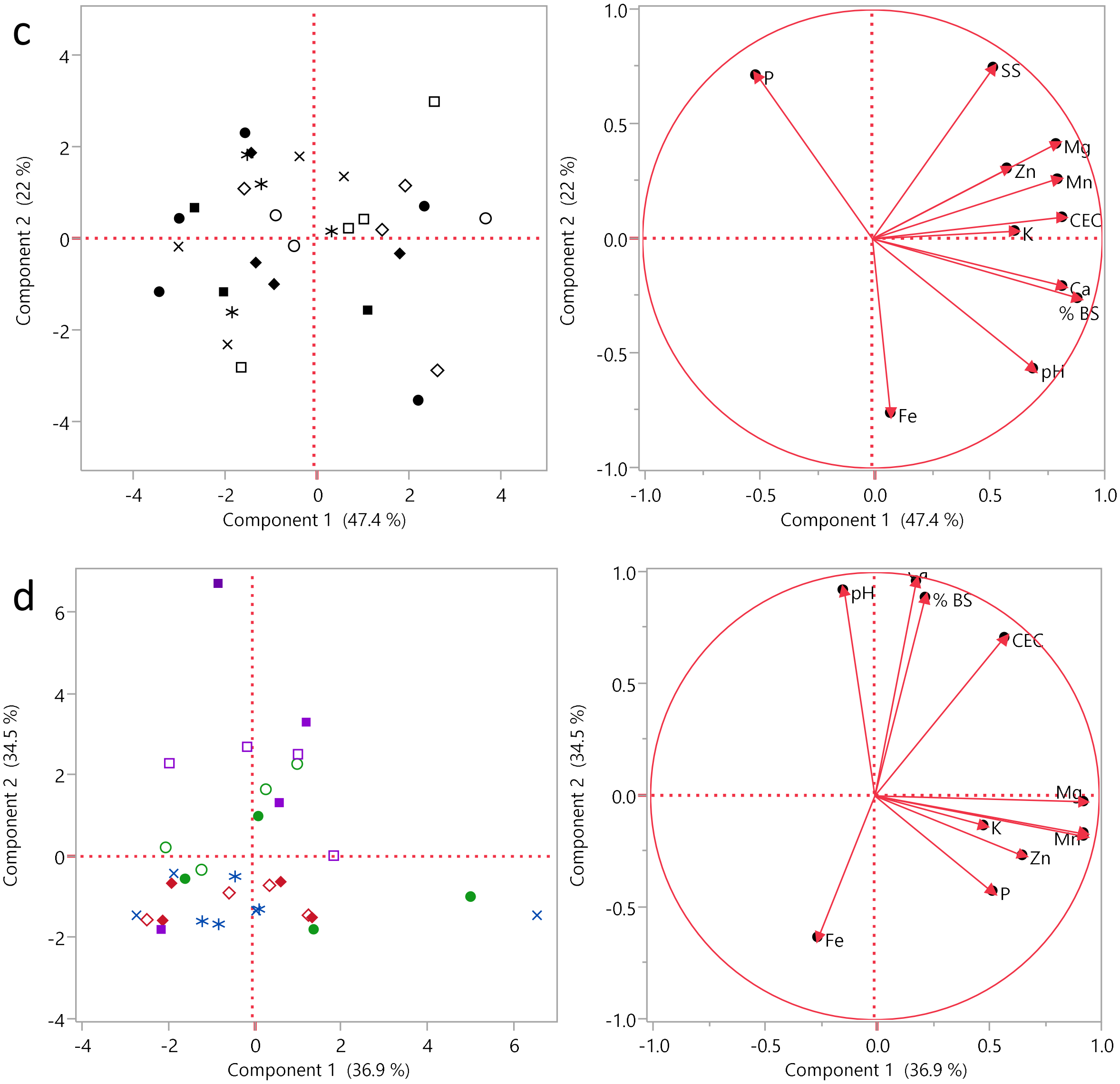


Figure 13. Principal component analyses (PCA) of soil chemical characteristics from the experimental plots at flowering (c) and harvest (d) times. The first and second principle components are plotted and the percentage of variance explained by each component is indicated within parenthesis on each axis. Closed symbols are raised bed treatments and open symbols are flat treatments. ◊: biochar; □: Shell; ○: Lime; x: Control.

4.3.3. Particulate Organic Matter and Soil C:N ratio

The carbon and nitrogen content of particulate organic matter are part of the labile organic fraction and are readily metabolized by soil microorganisms, which can lead to an increase of available nutrients for crops. In the 2014 growing season, only soil amended with biochar exhibited increased amounts of POM carbon and higher soil C:N ratios (Table 6). The increase was observed at transplant time and persisted through the harvest sampling. In contrast, lime and shell amendments did not significantly affect POM carbon or nitrogen. The planting method did, however, affect the labile organic compounds, with raised beds having increased POM C and reduced POM N at transplanting. This effect of planting method was not observed at harvest.

Table 6. Summary of two-way ANOVA results of the influence of amendments, planting method and sampling time on POM C (mg g^{-1}), POM N (mg g^{-1}), and C:N ratio.

	Transplanting			**Harvest**		
Source of variation	POM C (mg/g)	POM N (mg/g)	C:N	POM C (mg/g)	POM N (mg/g)	C:N
Amendment	0.0006	ns	<.0001	0.0092	ns	0.0005
planting method	0.026	0.0352	ns	ns	ns	ns
*Amend*plant method*	ns	ns	ns	ns	ns	ns
	Means comparisons					
Control	1.83 b	0.10 a	14.2 b	1.85 b	0.09 a	14.6 b
Biochar	2.61 a	0.10 a	17.7 a	3.14 a	0.11 a	16.6 a
Lime	1.65 b	0.08 a	14.9 b	2.11 b	0.12 a	14.2 b
Shell	1.88 b	0.09 a	15.2 b	1.90 b	0.12 a	14.3 b
Flat	1.82 b	0.10 a	15.3 a	2.22 a	0.11 a	14.8 a
Raised Bed	2.16 a	0.08 b	15.7 a	2.28 a	0.10 a	15.1 a

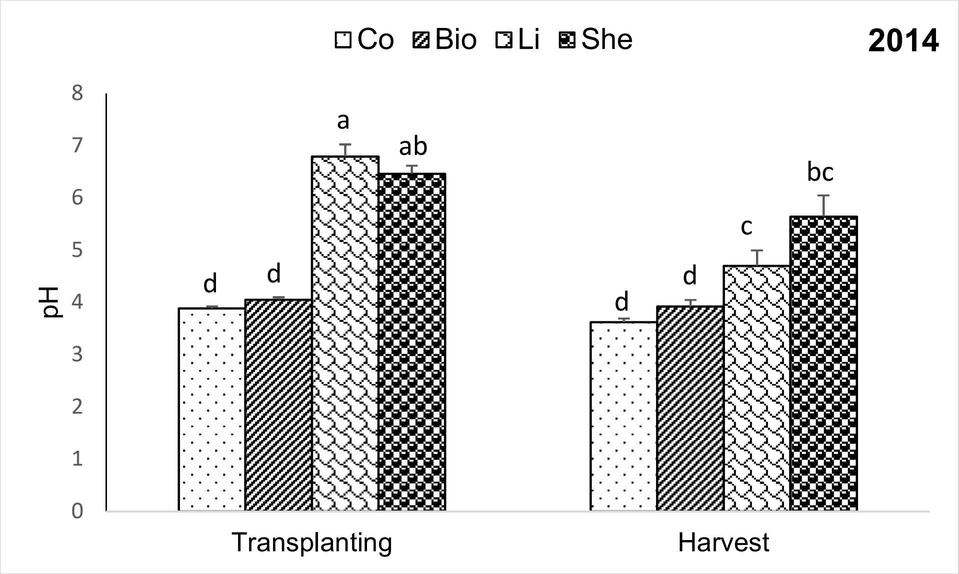
4.3.4 Effects of treatments on pH

The application of shell and lime significantly increased the mean pH of the soil from an initial value of 3.8 to 6.46 and 6.79, respectively, at transplanting time in the 2014 season ($p<0.05$) (Figure 14). At harvest, the mean pH had decreased to 4.69 in the lime treatment and 5.64 in shell, but those values were still significantly higher than the control and biochar treatments. Biochar plots were not significantly elevated in pH over the control plots. In 2015, the soil amendments were not reapplied and the amendment effects on pH were sharply decreased. In fact, only the shell plots maintained at a pH that was significantly ($p<0.05$) different from the control. All samples from lime and shell plots had lower pH than the first year at transplanting and harvest. The highest mean pH in 2015 was measured at transplanting and was 4.04 in the lime plots and 4.34 in the shell plots.

Therefore, the ability of the soil amendments to increase the pH clearly decreased in a single year. There was no significant effect of planting method on pH in either year (Table 7).

Table 7. Summary of repeated measure ANOVA results of the influence of amendments, planting method and sampling time on pH, available Al and Fe concentrations.

		2014			2015		
		pH	**Al$_{KCl}$**	**Fe**	**pH**	**Al $_{KCl}$**	**Fe**
Source of variation	**Df**	**p<0.05; *P<0.1					
Time	1	<0.0001**	0.0226**	<0.0001**	0.053*	0.084*	<0.0001**
Amendment	3	<0.0001**	<0.0001**	0.0005**	<0.0001**	0.056*	ns
Pl. Met.	1	ns	ns	ns	ns	ns	ns
Time*Amendment	3	<0.0001**	ns	ns	ns	ns	ns
Time*Pl. Met.	1	ns	ns	ns	ns	ns	ns
Amendment*Pl. Met.	3	ns	ns	ns	ns	ns	ns
Time*Amendment*Pl. Met.	3	ns	ns	ns	ns	ns	ns



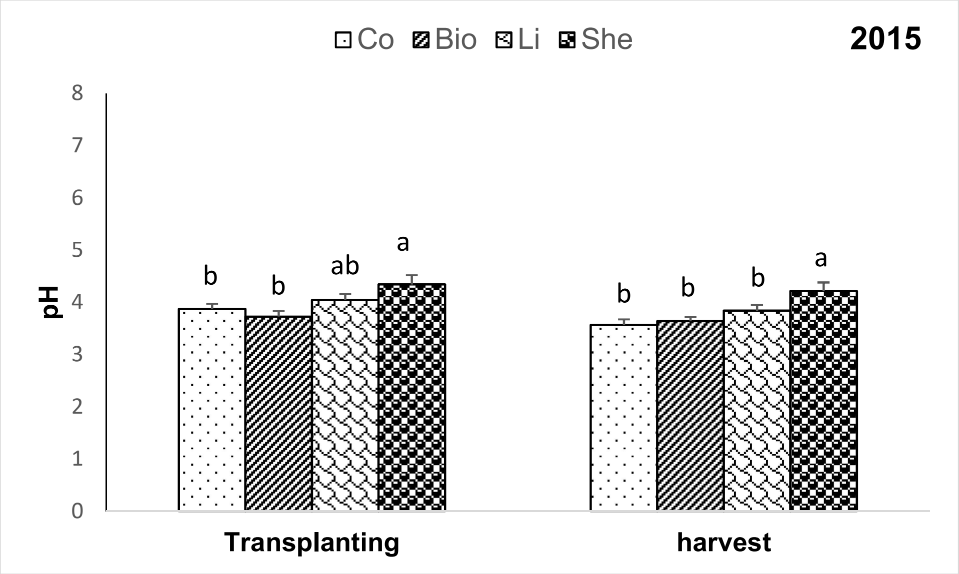


Figure 14. Mean soil pH values following each soil amendment at transplanting and harvest time in each growing year. $P<0.05$; Different letters denote significant difference.

4.3.5 Effects of soil amendment on soluble aluminum

The exchangeable aluminum form Al^{3+} can be toxic to harmful at high concentrations (Viera et al., 2008) and it is the predominant aluminum form at $pH < 4.5$, representing a primary source of toxicity to plants. The soil amendments significantly affected exchangeable Al^{3+} in 2014 with lower values in the shell and lime plots ($p<0.05$) (Figure 15).

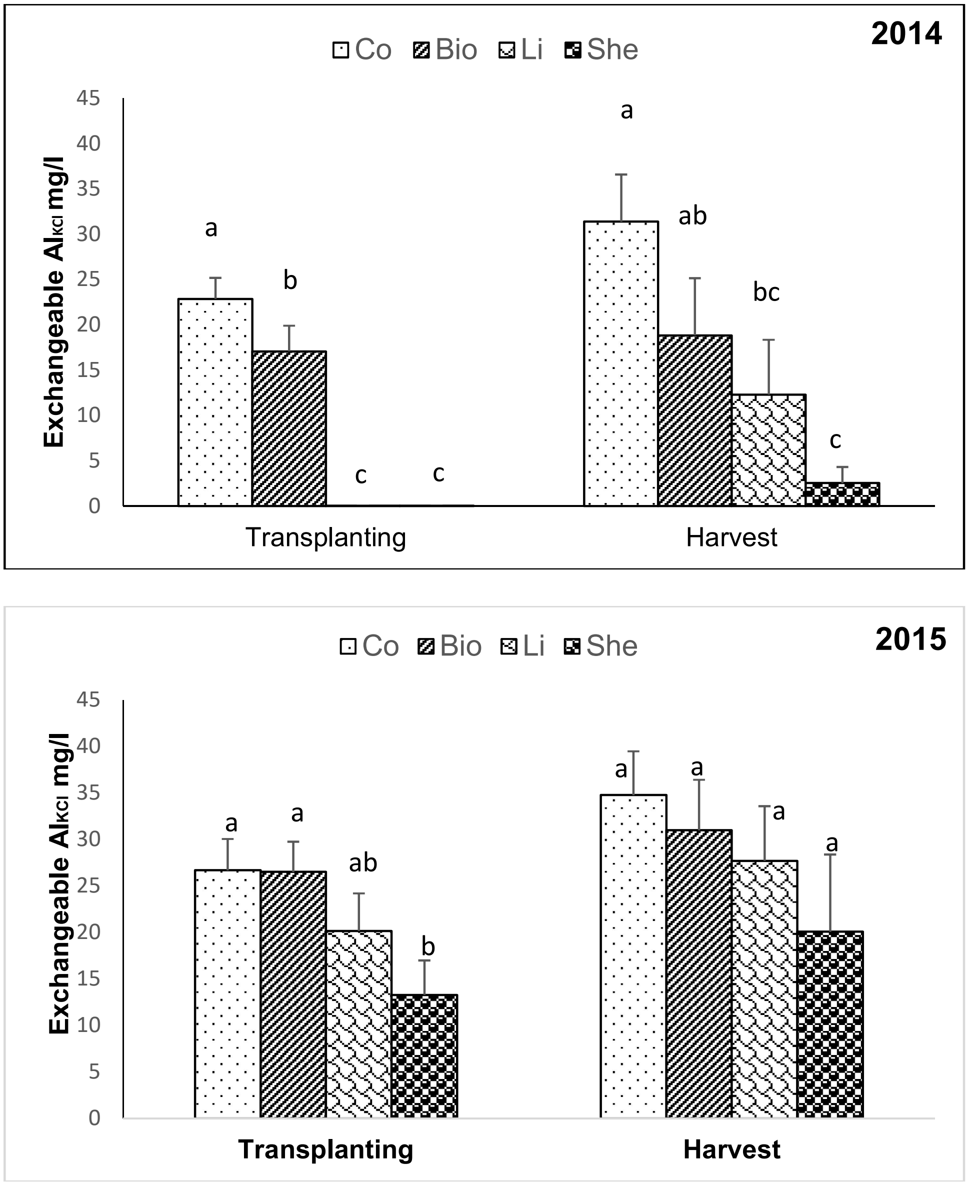


Figure 15. Mean concentrations of exchangeable Al^{3+} in soils from each treatment amendment at transplanting and harvest times for the 2015 growing season. Letters denote significantly different values ($p<0.05$ for 2014 and $p<0.1$ for 2015).

For example, at transplanting time, exchangeable aluminum was reduced by approximately 100%, while at harvest time the reduction was about 80%. Biochar also significantly decreased the presence of exchangeable aluminum but the reduction was less than with the shell and lime. The effect of biochar was significant only at transplanting. In 2015, only the shell treatment continued to significantly reduce Al concentrations at transplanting time, and there was no significant difference among treatments at harvest. The decrease is likely related to the increased pH since KCl (extractant reagent) is a nonbuffered solution. Our results showed a strong negative linear relationship existed at pH < 5 between exchangeable Al and pH for each year ($R^2=0.611$ $p<0.0001$ in 2014 and $R^2=0.648$ $p<0.0001$ in 2015). Exchangeable Al decreased in soils with pH up to 5.5 and was below detection at pH ≥5.5 (Viera et al., 2008) for both years (Figure 16).

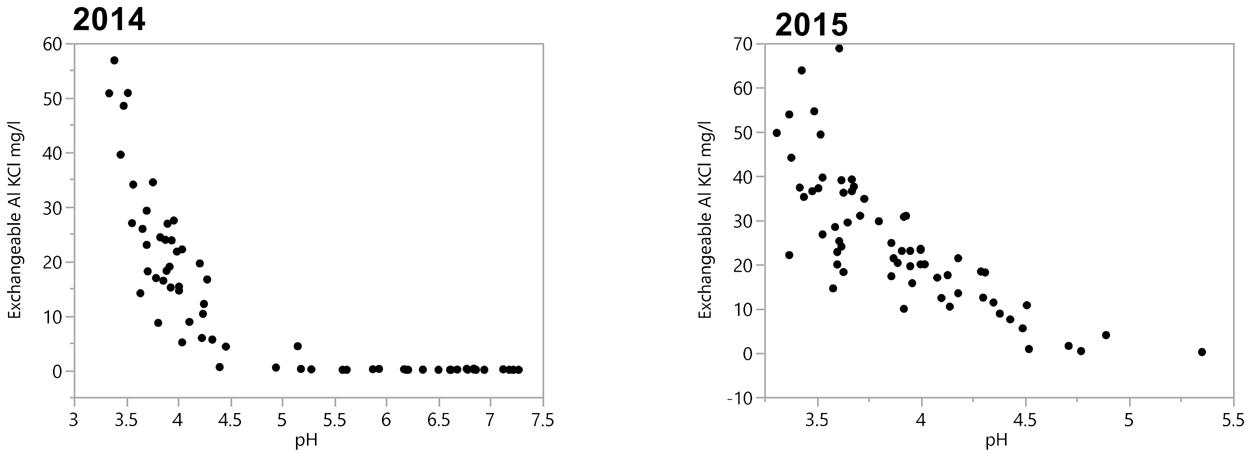


Figure 16. Relationship of soluble Al to soil pH in all samples combined for the 2014 and 2015 growing seasons. The values above pH 5.5 are below the detection limit (<0.017 mg L^{-1})

4.3.6 Effects of treatments on soluble Fe

Iron solubility is a key factor in determining its availability and uptake by plants and depends greatly of the soil pH. When pH increases (OH^- prevalent), Fe reacts with OH^- and precipitates in the form of $Fe(OH)_3$ (Fageria et al., 2008) or other oxy-hydroxides. Acidification will cause the release of soluble Fe^{3+} ions (not easily taken up by plants) that can become Fe^{2+} (readily available for plants) under reducing conditions (Weber et al., 2006). Increasing Fe^{2+} concentrations also inhibits the uptake of other nutrients, which can lead to nutrient disorders described by Fageria et al. (2008) and induce indirect toxicity that can damage plants. Measurement of Fe concentrations during the first year growing season

(2014) showed there was no effect of planting method but there was an effect of soil amendment (Figure 17; Table 7). Specifically, shell and lime amendments significantly reduced ($p<0.05$) Fe content to 70 mg L^{-1} and 78 mg L^{-1}, respectively, from 110 mg L^{-1} in control plots. At harvest, Fe concentrations increased significantly in all of the amendments and the decreased concentrations in the shell and lime plots were no longer significant compared to the control. During the second growing year, only a time effect was observed with an increase of Fe concentrations from transplanting to harvest. There was no significant difference among the amendments applied the year before. Linear regression (Figure 18) indicates a strong relationship between Fe and pH in 2014 ($R^2=0.316$ and $p<0.0001$) but no relationship in 2015. A strong relationship was observed (Figure 18) between exchangeable Fe and total Fe determined by acid digestion ($R^2=0.168$ and $p=0.0008$ for 2014 and $R^2=0.485$ and $p<0.0001$ for 2015).

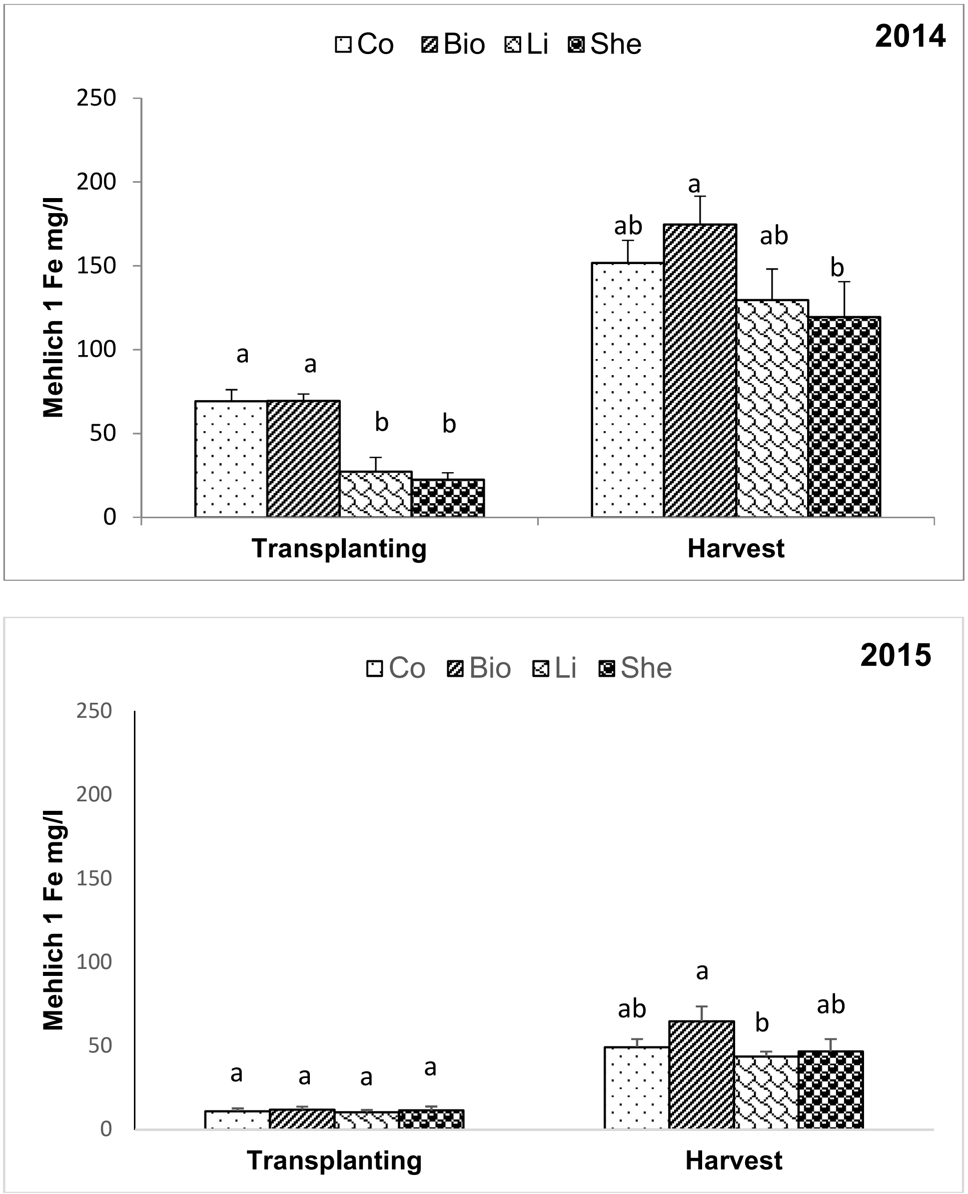


Figure 17. Mean concentrations of extractable Fe (Mehlich 1) in soils from each treatment amendment at transplanting and harvest times for the 2014 (top) and 2015 (bottom) growing seasons. Letters denote significantly different values ($p<0.05$).

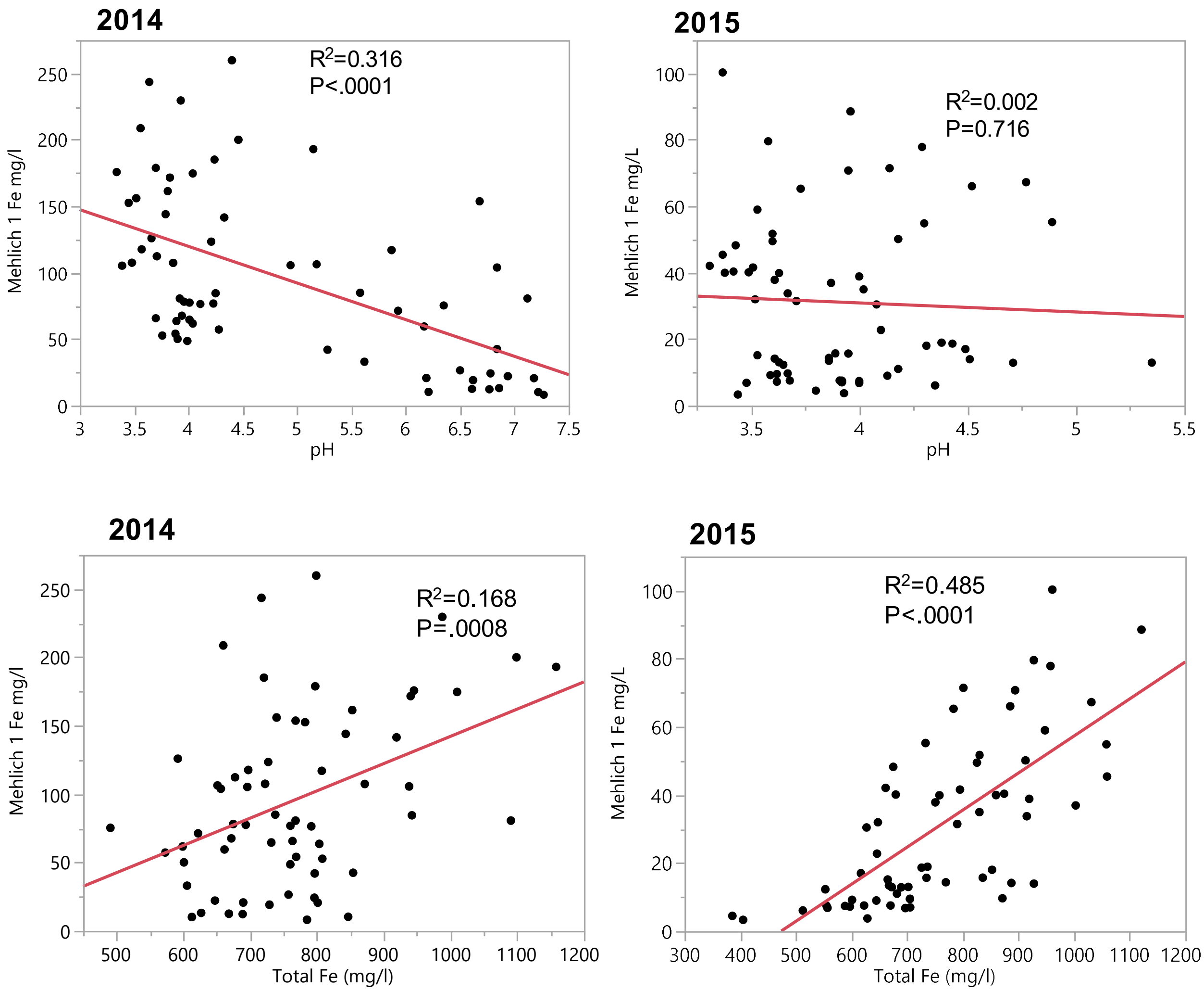


Figure 18. Simple linear regression and correlation coefficient relationships between extractable Fe (Mehlich 1 Fe) and pH (top) and or total Fe obtained by acid digestion (bottom) for the two growing season years.

There was no significant difference in the total Fe concentrations obtained by acid digest among the different treatments. Therefore, it is reasonable to assume that the changing level of exchangeable Fe among treatments is related to differences in Fe availability impacted by the applied soil amendments.

4.3.7 Effects of treatments on Fe fractions

Iron is present in soil in a number of different forms that determine its availability to plants. Therefore, the determination of total Fe does not give information about its mobility and lability. For example, Fe accumulates in soil in forms such as water soluble, exchangeable, bound to carbonates, bound to organic matter, bound to oxides, and directly as part of minerals (Tessier et al., 1979). Water soluble and exchangeable fractions are readily available to plants, while the less labile fractions (carbonates, organic, oxides) are considered only potentially available and depend on physical-chemical characteristics of the soil (Okoro et al., 2012). The last residual fraction is generally considered unavailable (Walna et al., 2010).

The determination of Fe fractionation throughout the depth of the soil profile revealed variability in Fe forms among horizons (Figure 19). The concentrations of water soluble and exchangeable Fe were low but increased with depth. Carbonate and organic bound Fe were predominant in the upper horizons, which could be available to plants with a change in the redox or pH conditions. The crystalline oxides fraction represented the highest proportion in the lower layers of the profile. The pyrite concentration increased with the depth and was higher in the two lowest layers that corresponded to the parent material. Its formation at this depth could be linked to the high concentration of sulfur and Fe observed in those layers (Appendix 1). In fact, pyrite formation requires available organic matter, sulfate, sulfur reducing bacteria, and Fe (Claff et al., 2010). The sum of all Fe fractions was higher on the surface (0.8 g kg^{-1}) compared to medium layers and rose to more than 1.1 g kg^{-1} in the lower part of the soil profile (Table 8). In terms of bioavailability or lability, the upper horizons were more susceptible to release of Fe because their lowest proportion of Fe was in fractions in that are poorly available (Fe crystalline-oxides) or unavailable (Fe-residuals) (Walna et al., 2010). This latter fraction remained low through the soil profile.

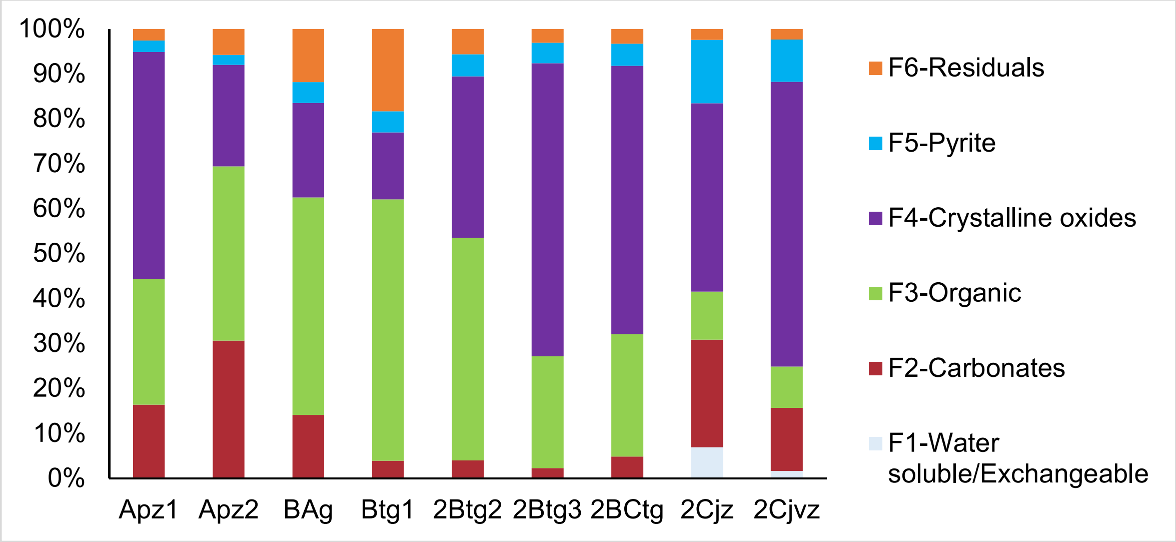


Figure 19. Percentage of Fe in each fraction determined from a 6 step sequential extraction in each horizon of the soil profile. Percentages shown represent the means of triplicate analyses (the deviation between duplicate samples was less than 8%).

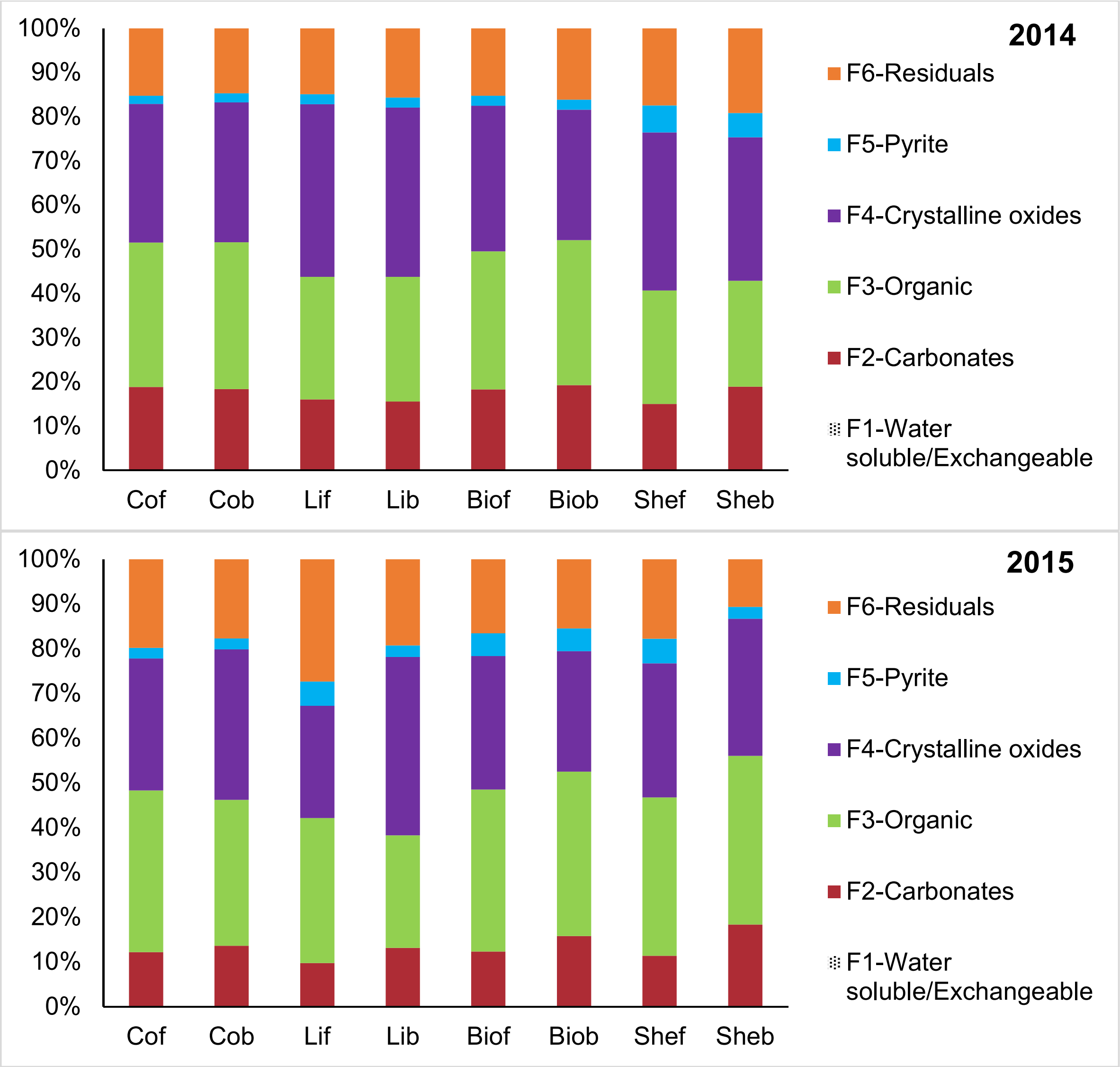


Figure 20. Percentage of Fe in each fraction determined from each phase of a 6 step sequential extraction of soil samples from the experimental plots (0-10 cm) at transplanting time in 2014 (top) and 2015 (bottom). Percentages shown represent the means of four replicate analyses (the deviation between replicates samples was less than 8%).

Iron concentrations of each individual fraction determined by the sequential extraction procedure are given in Figure 20 and Table 8 for the experimental plots at transplanting time during the 2014 and 2015 seasons. Iron recovery was determined by comparing the amount of Fe recovered in individual fractions to a total Fe extraction. Iron recovery from sequential extraction analyses were within ±10% for 2015 samples and ±25% for 2014 of the independent total Fe concentrations obtained by the HNO_3:HCl digestion method. The water soluble/exchangeable fraction was the lowest percentage (<1%) of the total Fe. In both years, organic bound Fe and crystalline Fe oxides represented the highest proportions of total Fe. Their cumulative total represented more than 60% of the Fe extracted in all steps. The difference between these two fractions is their ability to release the Fe. In 2014, Lime and Shell treatments had the highest proportion of Fe bound in fractions that are poorly available or unavailable (crystalline Fe oxides and residuals). Therefore, they represent a reserve (Mescouto et al., 2011) that is more stable. There were no significant differences in the sum of Fe fractions extracted among the treatments.

Table 8. Iron (mg/L) concentrations of the soils (mean±SE, n=4) within each defined chemical fraction of a sequential extraction.

	H_2O+Exch.	Carbonates	Organic	Cryst/oxides	Pyrite	Residuals	Sum	Total Fe [a]
Soil profile								
Apz1	1.70 ± 0.02	122 ± 4.7	209 ± 2.2	377 ± 1.7	19.2 ± 0.26	20 ± 2.1	749 ± 7	866.9 ± 0.88
Apz2	0.72 ± 0.01	153 ± 2.4	194.6 ± 0.96	113 ± 1.8	10.9 ± 0.21	29.2 ± 0.97	502 ± 5.6	625 ± 1.8
BAg	0.24 ± 0.00	28.2 ± 0.42	98 ± 5.1	42.56 ± 0.06	9.3 ± 0.69	24 ± 1.9	202 ± 3.7	307.2 ± 0.39
Btg1	0.20 ± 0.00	5.2 ± 0.15	84 ± 16	20 ± 3.4	6.6 ± 0.19	26 ± 1.9	143 ± 15	268 ± 1.6
2Btg2	0.88 ± 0.02	16.9 ± 0.12	222 ± 3.9	161 ± 3.5	22± 3.02	26 ± 4.3	449 ± 5.7	710 ± 17
2Btg3	0.48 ± 0.01	24.2 ± 0.42	272 ± 9.8	714 ± 2.0	49 ± 9.5	34 ± 1.9	1094 ± 2.9	1342 ± 46
2BCtg	1.21 ± 0.04	61.7 ± 0.67	353 ± 21	773 ± 23	64 ± 3.4	43 ± 2.8	1296 ± 45	1423 ± 20
2Cjz	76.1 ± 0.71	265 ± 2.0	118 ± 2.1	463 ± 10	156± 3.5	27 ± 3.5	1106 ± 19	1137 ± 4.5
2Cjvz	31 ± 1.06	265 ± 11	173 ± 9.7	1194 ± 35	178 ± 11	44 ± 4.3	1886 ± 66	1763 ± 177
2014								
Cop	0.29 ± 0.03	97 ± 11	167 ± 12	161 ± 12	9.6 ± 0.22	77 ± 5.4	513 ± 25	784 ± 38
Cob	0.25 ± 0.02	84 ± 8.7	151 ± 8.5	146 ± 15	9.3 ± 0.99	68 ± 9.5	460 ± 32	742 ± 23
Lip	0.0 ± 00	89 ± 10	152 ±5.6	220 ± 34	12.1 ± 0.89	81 ± 4.3	55 ± 45	812 ± 100
Lib	0.02 ± 0.01	76 ± 8.6	138 ± 6.0	188 ±7	11.1 ± 0.82	77 ± 6.6	492 ± 27	748 ± 45
Bip	0.22 ± 0.01	90 ± 12	153 ± 12	160 ± 8.6	10.9 ± 0.99	74 ± 8.5	489 ± 28	777 ± 70
Bib	0.23 ± 0.01	91 ± 7.8	155 ± 8.5	139 ± 5.2	10.5 ± 0.69	76 ± 7.9	472 ± 19	699 ± 41
Shep	0.01 ± 00	71 ± 13	125 ± 17	178 ± 30	30 ± 13	85 ± 12	490 ± 61.	770 ± 47
Sheb	0.01 ± 00	90 ± 11	106 ± 22	151 ± 9.3	30 ± 21	95 ± 30	472 ± 42	669 ± 32
2015								
Cop	0.25 ± 0.05	75 ± 12	220 ±16	180 ± 14	14.3 ± 0.90	119 ± 7.3	639 ± 32	635 ± 38
Cob	0.35 ± 0.02	95 ± 8.9	227 ± 16	235 ± 23	17.1 ± 0.62	122 ±	697 ± 41	747 ± 63
Lip	0.27 ± 0.03	70 ± 4.0	241 ± 27	190 ± 37	39 ± 9.5	210 ± 48	752 ± 104	681 ± 84
Lib	0.25 ± 0.05	91 ± 11	174 ± 20	280 ± 19	18 ± 1.5	134 ± 14	698 ± 20	665 ± 18
Bip	0.27 ± 0.05	89 ± 21	239 ± 27	206 ± 38	36 ± 11	113 ± 20	684 ± 109	666 ± 94
Bib	0.29 ± 0.06	106 ± 15	243 ± 22	177 ± 23	36 ± 14	106 ± 30	670 ± 70	682 ± 46
Shep	0.29 ± 0.17	62 ± 6.3	197 ± 24	170 ± 23	35 ± 15	104 ± 30	571 ± 80	540 ± 61
Sheb	0.15 ± 0.05	124 ± 12	257 ± 16	210 ± 22	17.7 ± 0.82	71 ± 8.4	681 ± 28	690 ± 17

[a]independent total concentration which resolved by the HNO_3-$HClO_4$ digestion method

4.3.8 Effects of treatments on soil microorganisms: total bacteria and Geobacter

Geobacter are Fe-reducing bacteria that use Fe^{3+} as an electron acceptor and reduce it to Fe^{2+} under anoxic conditions. Their role in facilitating the transition between these two forms of Fe is important in the overall biogeochemistry of acidic environments, and the abundance of *Geobacter* depends on pH (Weber et al., 2006). Analysis of *Geobacter* concentrations in experimental plots (Table 9) indicated a significant time x amendment interaction and a higher abundance over time in soil amended with shell (Figure 21). The results further indicate that the shell effect was more pronounced on soils collected at transplant time (1.6×10^7 copies g^{-1} soil). Biochar had no effect on *Geobacter* and exhibited the lowest populations. The abundance of *Geobacter* in shell and lime treatments can be explained by the higher pH recorded in those treatments. At high pH, Fe^{3+} is more present than Fe^{2+} in the soil solution and therefore constitutes more substrate to support these populations. As shown in Figure 22, evaluating *Geobacter* abundance and pH with linear regression yielded a significant relationship with an $R^2 = 0.217$ ($p=0.0008$). There was no difference among the treatments in total bacterial abundance as measured by concentrations of 16S rRNA gene copies, but their abundance did increase from transplanting to harvest regardless of the type of amendment applied. The ratio of *Geobacter* to total bacteria was higher at transplanting time particularly in the shell treatment compared to the control.

Table 9. Summary of repeated measurement ANOVA results of the influence of amendments, planting method and sampling on abundance of total bacteria, Geobacter, and ratio Geobacter/total bacteria ($p<0.05$).

Source of variation	**df**	***Geobacter***	**Total Bac**	**Ratio**
Time	1	ns	< 0.0001	0.0044
Amendment	3	0.0003	ns	0.015
Planting Method	1	ns	ns	ns
*Time*Amendment*	3	0.027	ns	ns
*Time*Planting Method*	1	ns	ns	ns
*Amendment*Planting Method*	3	ns	ns	ns
*Time*Amendment*Planting Method*	3	ns	ns	ns

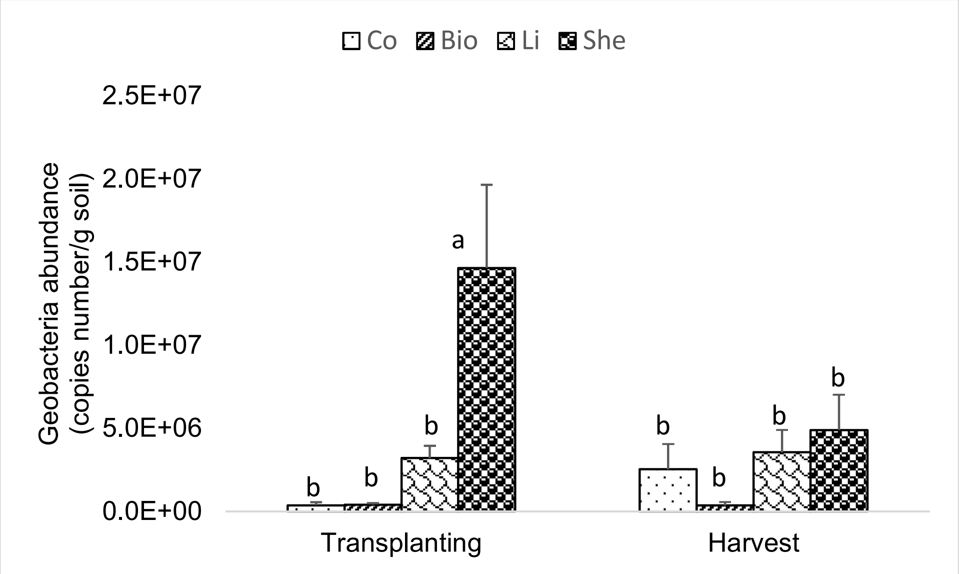


Figure 21. *Geobacter* gene copy abundance for each soil treatment at transplanting and harvest time for the 2014 growing season. Treatments with different letters are significantly different (p<0.05).

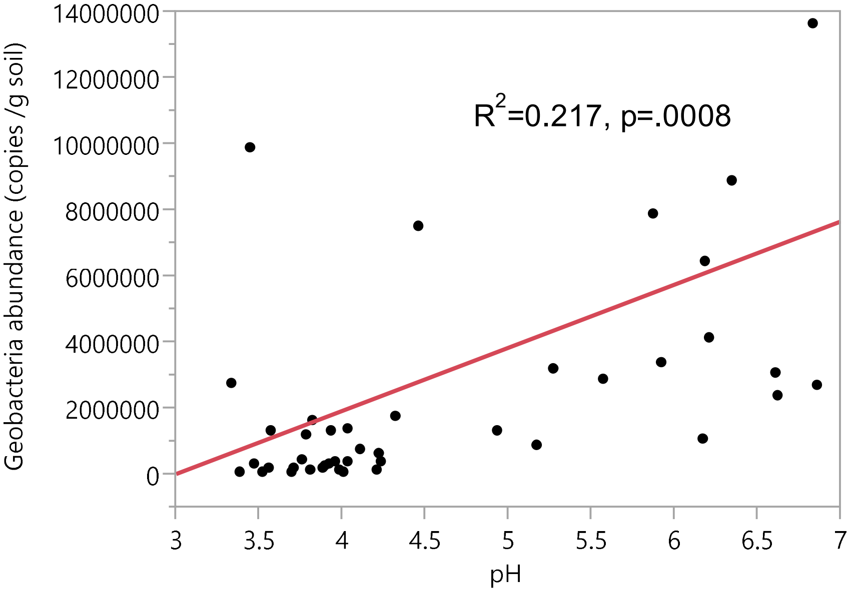


Figure 22. Simple linear regression and correlation coefficient relationships between *Geobacter* gene copy abundance and soil pH for the 2014 growing season.

4.3.9 Effects of soil amendments on rice yield

Soil amendment treatments had significant impact on rice yield parameters in both growing seasons. In 2014, the plots treated with shell and lime recorded the highest values of tillers hill^{-1}, panicles m^{-2}, 1000 grain weight, height, and yield (Table 10). In 2015 there was a decrease in those parameters and only the shell treatment was significantly different from the control for parameters of tillers hill^{-1}, 1000 grain weight, and grain yield. There was a strong positive relationship (Figure 23) between yield (t ha^{-1}) and the pH in both years ($R^2=0.312$; $p=0.0009$ and $R^2=0.474$ $p<0.0001$). Planting method had no significant effect on plant production or yield (Table 10).

Table 10. Summary of two-way ANOVA results of the influence of amendments, planting method and sampling on yield parameters.

2014						
Source of variation	**df**	**Tillers/hill**	**Panicles/m^2**	**1000 grains weight (g)**	**Height (m)**	**Grain Yield (t /ha)**
Amendment	3	0.007**	0.053*	0.008**	0.004**	0.061*
Planting method	1	ns	ns	ns	ns	ns
*Amend*planting*	3	ns	ns	ns	ns	ns
	Means comparisons					
	She	15.0 a	166.0 a	25.12 a	0.96 a	1.8 a
	Li	11.12 ab	132.75 a	21.25 a	0.76 a	0.98 ab
	Bio	8.1 ab	90.0 ab	16.0 ab	0.57 ab	0.67 ab
	Co	4.02 b	25.0 b	6.12 b	0.20 b	0.29 b
2015						
Source of variation	**df**	**Tillers/hill**	**Panicles/m^2**	**1000g weight (g)**	**Height (m)**	**Grain Yield (t /ha)**
Amendment	3	0.008**	ns	0.059*	ns	0.075*
Planting method	1	ns	ns	ns	ns	
*Amend*planting*	3	ns	ns	ns	ns	
	Means comparisons					
	She	9.37 a	130.0 a	25.2 a	0.66 a	0.73 a
	Li	7.12 a	127.12 a	21.3 a	0.54 a	0.49 ab
	Bio	6.0 ab	86.0 a	16.0 ab	0.60 a	0.45 ab
	Co	3.18 b	39.87 a	6.1 b	0.46 a	0.15 b

(**$p<0.05$, *$p<0.1$); Different letters denote significant differences within each column of response variables.

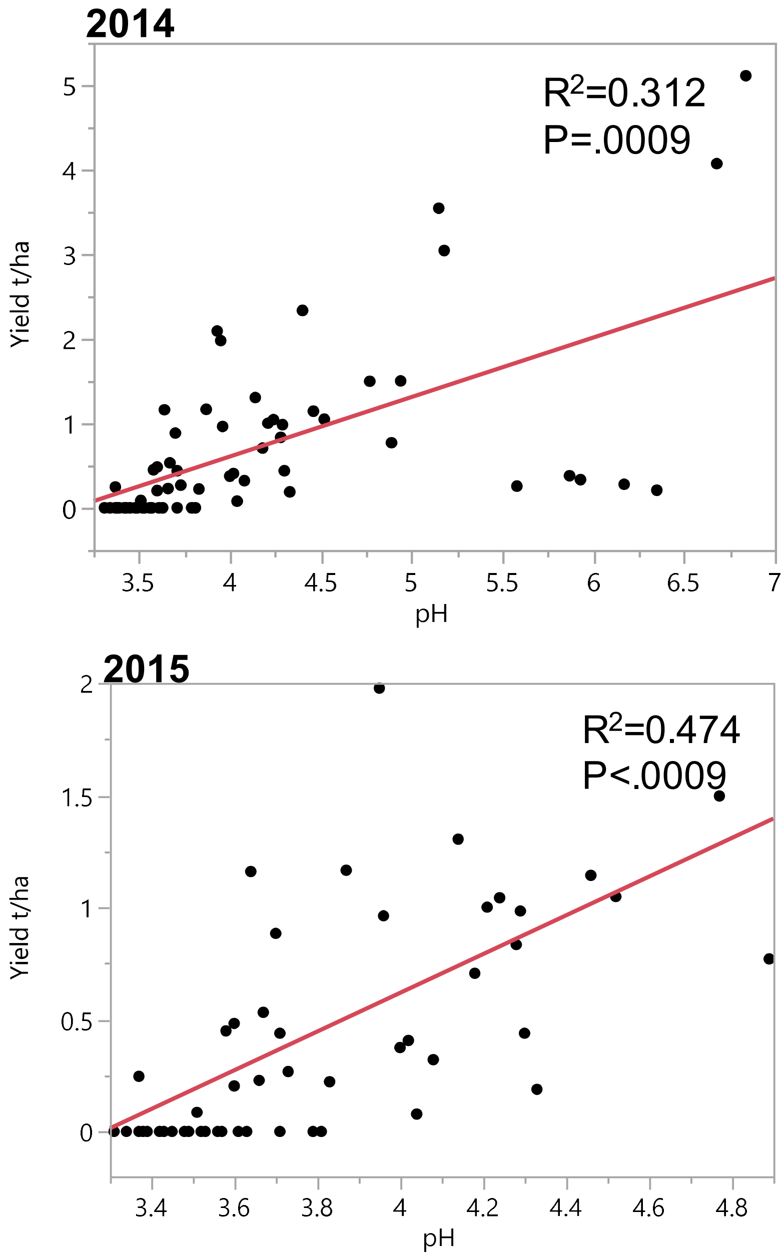


Figure 23. Simple linear regressions between yield (t ha^{-1}) and soil pH for 2014 (top) and 2015 (bottom).

5. DISCUSSION

To meet food demands in Senegal, improvement and rehabilitation of rice paddies by developing specific strategies for acidity, nutrient toxicities, and poor soil fertility in rice growing areas are necessary. The description of the soil profile from Djibelor confirmed the presence of an acid sulfate soil with low pH and low P availability and also potential occurrence of Fe and Al toxicities (Shazana et al., 2013). In order to achieve high productivity in the soil, it will require mitigation with appropriate amendments and agronomic practices. Additionally, productivity will depend on climate and rainfall variability. During the two years (2014 and 2015) of this study, precipitation was greater than the mean annual of 1200 mm (1390 mm and 1435 mm respectively), which increased the water availability for hydrological and agricultural purposes. This is important because the cultivation of rice in Casamance lowlands is subject to rainfed conditions and depends on the flooding of the surface and the rising of the ground water level above the soil surface before transplanting rice seedlings in August (figure 10). Therefore, rainfall can have broad consequences including available cropping area, growth, and yield. This is particularly important at the early growth stage, when availability of water can be determinant and have consequences on overall rice yields (Koide et al., 2013). In our study, water availability did not appear to be a limiting factor and, given that light and temperature are not limiting for rice production in this area and no fatal weather events were recorded, we were able to detect significant differences in soil characteristics, plant growth, and yield as a result of the amendment treatments. However, it is important to note that in future years with significant water limitations, soil amendments alone may not be sufficient to realize significant gains in rice productivity.

5.1 Amendment effects on soil properties and yield

Liming has significant effects on nutrient availability and is a useful tool to restore acidic soils and improve agricultural yields (Lee et al., 2008). When lime is added to the soil, cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) replace hydrogen ions H^+ on the soil exchange complex. In addition, the CO^{3-} anions react with soil protons (H^+) to neutralize and decrease acidity. In this study, the use of shell and lime caused a significant increase in pH, but this was not the only beneficial effect. The strong relationship between pH and available nutrients, base saturation, and yield illustrates the interdependence among all of these factors in soil fertility (Adams, 1984). This explains the results observed in this two-year experiment, particularly the first year (2014), in which the plots amended with liming materials (shell and lime) recording the highest pH, highest exchangeable cations and nutrients, highest yield, and lowest potentially toxic forms of Al and Fe.

Shell and lime as liming materials are rich in Ca and, therefore, increase the availability of exchangeable Ca. In our treatments this resulted in significantly higher Ca ($p<0.05$) and this was accompanied by higher CEC and base saturation, revealing a modification in the soil exchange complex. Firstly, the interactions of Ca^{2+}, H^{+}, and CO_3^{-} reacted as described above to neutralize acidity and raise pH. However, the interactions between factors in soil could also explain the changes in Al^{3+} availability, which decreased significantly following shell and lime amendments in our study. This decrease is especially important as it eliminated risk of Al^{3+} toxicity, which is a common stress factor in acidic soil with $pH < 4$ (Ryan and Delhaize, 2010; Shamshuddin et al., 2013). Although Al concentrations can vary at a given pH value, overall Al^{3+} concentrations tend to decrease with increasing pH (Adams, 1984). The increased pH induces Al precipitation as Al hydroxides in the root system, where plants are particularly susceptible, and reducing its availability to crops and toxicity (Ryan and Delhaize, 2010). This is why after determination of pH (<3.5) in the soil prior to the Djibelor experiment, the amendment rates were calculated to target the optimum pH for crops and to also alleviate the potential occurrence of Al toxicity.

Djibelor soil can be defined as high fertility paddy soil with 2.7% organic matter and 0.11% nitrogen (Jia-fang and Shi-ye 1981). From the experiment, no liming effect was observed on POM or soil organic carbon (Wyngaard et al., 2012; Hati et al., 2008). Changes on these parameters are linked to activity of microbial communities that play important roles in soil function. For example, soil microorganisms impact nutrient availability and plant health and are sensitive to soil or environmental conditions. In our study, lime increased C mineralization rates more than control and biochar. This stimulation of biological activity is often detected as an increase in soil organic matter mineralization or CO_2 emission. This results in acceleration of the organic matter turnover and smaller soil organic carbon stocks

(Paradelo et al., 2015). However, liming effects on soil organic carbon content can be variable. It could result in decrease (Chan and Heenan, 1999) or an increase (Briedis et al., 2012) in the soil organic carbon content relative to biomass productivity that could make changes in soil organic matter pools and carbon sequestration.

The improved soil chemical and biological properties resulted in increased crop productivity, particularly for the first growing year (2014). Shell and lime treatments produced the highest yields and this is hypothesized to have resulted from enhanced uptake of nutrients compared to control and biochar-amended plots. This is in agreement with the results obtained from Fageria et al. (2010), who studied the response of common bean (*Phaseolus vulgaris* L.) to lime and Fe applications in an acid soil over three years. They observed significant yield increases as a result of amending with lime only and also observed positive associations with soil pH, calcium, magnesium, hydrogen + aluminum, base saturation, acidity saturation, cation exchange capacity, and ratios of Ca:Mg, Ca:K and Mg:K. These results support the benefits of using limning agents to manage soil acidity and fertility through increased availability of essential nutrients for rice growth in Casamance.

The chemical composition of biochar is an important factor in determining how it will affect soil processes after amendment. For example, the composition of feedstock determines its stability, which is considered a critical factor in ensuring benefits to soil characteristics (Budai et al., 2014). Our results showed that biochar as an input for Djibelor soil did not significantly influence soil CO_2 emission during the laboratory incubation period. This is consistent with Lu et al. (2014) and Zhang et al. (2012), who also observed an absence of changes in CO_2 emissions following biochar amendment in a laboratory incubation and in a two-year rice experiment. Typically, biochar is relatively stable in soil and results in slower organic mineralization rates, leading to a reduction of the CO_2 emissions, which is referred as a negative priming effect (Lu et al., 2014). This resistance of biochar to biological degradation is due to its high molecular weight and reduced external/exposed functional groups. In our study, the lack of an effect of the biochar could be linked to the increase of C:N ratio. In fact, we did observe an increase in POM C, which represents an input of an energy source. However, the increase in C:N ratio indicates reduced nitrogen N availability, which can limit the decomposing microorganisms and slow mineralization. In the field, N availability in paddy soils is related to mineralization processes from soil and organic matter, which is why the use of biochar rich in soil nutrients is advocated in these systems. Beneficial sources can include manure-derived biochar (Cantrell et al., 2012), wastewater sludge, and algal biomass that contains high N, P, K to ensure carbon sequestration and soil amelioration.

The pH of the biochar also plays an important role in determining its effects on soil since its application often leads to an increase in the overall pH. The liming effect following biochar application has been previously reported in other systems (Lehmann et al., 2011) and could help reduce the bioavailability of toxic forms of Al and Fe. In our study, however, properties such as pH and ion availability were not modified by biochar. In contrast, Manickam et al. (2015) used two rates of rice husk biochar and showed an increase of pH in a sandy acid sulfate soil that resulted in alleviation of plant root stress by aluminum and increased CEC. The yield in their study was also significantly increased. Even though biochar did not have significant effects alone in our study, beneficial effects in the Djibelor soil could be expected since a mix of biochar plus shell did show an increase in soil pH and a reduction of Fe concentrations during the laboratory incubation period. Therefore, an increase in nutrient uptake and plant development could potentially occur. For example, Qayyum et al. (2015) found that biochar mixed with compost reduced P sorption and increased P bioavailability. In Casamance, if a locally available feedstock could be used to increase sustainability of crop production, further research should focus on the appropriate feedstock to achieve benefits that have been seen in other studies as well as determining the most effective combination of biochar plus complementary amendments (e.g., biochar + shell or biochar + N) to provide increased pH and nutrients.

5.2 Residual effects of lime on soil properties and yield

Liming materials are advocated in acid soils to increase soil pH, eliminate metal (Al and Fe) toxicities, and increase nutrient availability, which are all expected to result in increased crop yield. However, unlike soluble fertilizers, the results of liming are typically not expected to be immediate after application (Follet et al., 1981). In temperate regions, the duration of effectiveness for one application is usually 3 to 5 years. Fettel et al. (2007) applied lime at six rates ranging from 0-4 t ha^{-1} on a soil from western New South Wales sown with peas (*Pisum sativum*). Their results showed an immediate effect that lasted for the duration of the four cropping seasons. However, in the tropics, small rates applied more frequently are usually more efficient (Follet et al., 1981).

The rate at which liming materials react with the soil solution depends on the size and the type of chemical compounds. The shell used in the field (Djibelor) had a neutralizing value (% Calcium Carbonate Equivalent; CCE) of 102%. Sieving through 20, 60, 100 % mesh gave % CCE values of 83.6%, 48.7, and 38.8% respectively. Therefore, the effective neutralizing value (ENV) or effective CCE was calculated to be 61.9%. The ENV is the fraction of the CCE that will react with soil acidity in the first year of application (Black, 1992; Carey et al., 2006). It is based on the particle size distribution of the powdered shell that determines how much of the material can immediately neutralize soil acidity; i.e., the finer the material, the larger the surface area that will react with soil. Alvarez et al. (2010) evaluated the effects of particle size of a high Mg limestone and its application schedule (single or split) on an acid soil two years after liming. They found that plots with a single application and with the finest limestone recorded the highest pH, Ca, CEC, Mg, and lowest exchangeable Al. The plots with the coarsest limestone were similar to the control. The shell and lime used in the field experiment in this study caused an increase in pH, BS, Ca and a decrease in exchangeable Al and Fe, and resulted in yield increases during the first growing year. However, all of the beneficial effects observed in the first year decreased during the second year, particularly the pH (e.g., shell decreased from 6.79 to 4.34 and lime decreased from 6.46 to 4.04 between 2014 and 2015 transplanting).

In addition to the particle size of liming material, other factors that determine the soil pH change after lime application include soil pH, degree of mixing, crop removal, and N fertilization rates. At Djibelor, the liming materials were applied only in the first year and it reacted rapidly and effectively with soil acidity to raise the pH, while it usually takes several months to one year to see lime effects on pH. At harvest, a pH decrease was observed on shell and lime amended soils compared to transplanting. No soil pH test was conducted at the beginning of the second year to estimate the need of lime application, but the N fertilizer application was maintained, which can exacerbate soil acidity. In addition, crop residues were exported. Based on our results, it appears that the beneficial liming effects of shell and lime as fine materials did not last as long as expected and a more appropriate mix of lime particle size should be determined. The residual effect of coarse lime material is greater than fine lime material because large lime particles react slowly with soil acidity and tend to remain longer in the soil (Fageria and Baligar, 2008). Therefore, a mix of fine and coarse materials may be most beneficial to achieve short-term mitigation of affected soils while also providing longer lasting benefits for future years. In addition, the frequency of application should be considered in future research. A variety of factors including application methods, depth, timing, and frequency of liming are all important practices and should be optimized in concert for improving liming efficiency and crop yields on acidic soils (Fageria and Baligar, 2008).

5.3 Alleviation of iron toxicity in paddy fields

The sequential extraction was performed to quantify individual Fe fractions associated with different chemical species, as well as forms that are potentially mobile, bioavailable or unavailable. Overall, there was no significant difference in total concentration as determined by the sum of fractions. However, lime and shell treatments did give lower proportions of readily available Fe (water soluble/exchangeable) at transplanting time. This was also in agreement with the lowest Mehlich 1 values of Fe (33.35 and 21.42 mg l^{-1} in lime and shell respectively) which was used to estimate bioavailable Fe. The effects of Fe in soil and plant tissues are related to mobile fractions rather than to the total concentration and are strongly impacted by its bioavailability (Weber et al., 2006). The readily available fractions, in concert with clay type, redox potential and soil pH, are considered the major factors that determine the bioavailability.

In addition, Fe mobility is also impacted by environmental variables. As pH increases after shell or lime amendment, bioavailable Fe decreases. Iron is converted to less soluble forms, principally to oxyhydroxides like $Fe(OH)_3$ or Fe_2O_3. In this study, a strong negative relationship (R^2=0.316, p= <0.0001) between pH and bioavailable Fe occurred in the field plots, suggesting that liming helped reduce Fe availability to crops. However, this relationship was less evident in the second growing season (2015) although lower concentrations of Fe were observed in all treatments. It is important to remember that the pyrite fraction present in the soil produces acidity when exposed to the atmosphere and continues to release Fe ions that affect rice growth (Shamsuddin et al., 2013). This continued weathering of the parent material continues to supply Fe to the soil solution, and constitutes another factor in managing Fe toxicity. In our study, significant mortality (100% in some plots) was observed in these experiments, particularly in the control plots. A reduction in plant height and the number of tillers was also observed. As explained above, the mechanism for this effect is expected to be the low concentrations of nutrients such as potassium, phosphorus, calcium, and magnesium, which further increases Fe uptake, causing toxicity. The maximum concentration of Fe recorded for either year in the plots was below 200 mg kg^{-1}, which is less than the level of 300 mg kg^{-1} in soil solution (Elec et al., 2013) and plant tissues (Wells et al., 1993) that has been defined as 'toxic.' However, this can only be considered as a guideline given that the toxicity of a given concentration depends on rice cultivars (Audebert and Sahrawat, 2000)

6. CONCLUSIONS

Overall, this study suggests that appropriately managed shell amendment of acid sulfate soils in Casamance can significantly reduce potential metal toxicities and increase rice yields. Shell material alone or associated with organic compounds such as biochar was shown to play an important role in promoting increased pH, which helps reduce Al^{3+} and Fe bioavailability and results in beneficial effects for cation levels and exchange capacity in the soil solution and soil solids. There is a strong relationship between pH and exchangeable cations as well as between the reduction of Al and Fe concentrations and yield. This resulted in yields that were higher in shell amended plots for both years, even though the water pH dropped below 5 in the second year. This work also demonstrates that the addition of liming materials affects the physical and chemical properties of soil and rice productivity. The positive effect of organic inputs on soil properties observed in laboratory controlled trials was verified in field experiments. Finally, it is important to note that the strong response to shell amendment was obtained during a normal year of rainfall. However, rainfall could still be a limiting factor and affect production in deficit years in the region. These results highlight the need for further research to better understand the effects of the timing and rate of soil amendments on yield predictions as they interact with rainfall variability and water management to affect rice production systems. In addition to the use of Fe-tolerant rice varieties, improved soil management holds promise as the best chance for improving rice yields. Results from this work can potentially be used to recover currently unproductive lands that are necessary to meet food demands of the Senegalese population.

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APPENDICES

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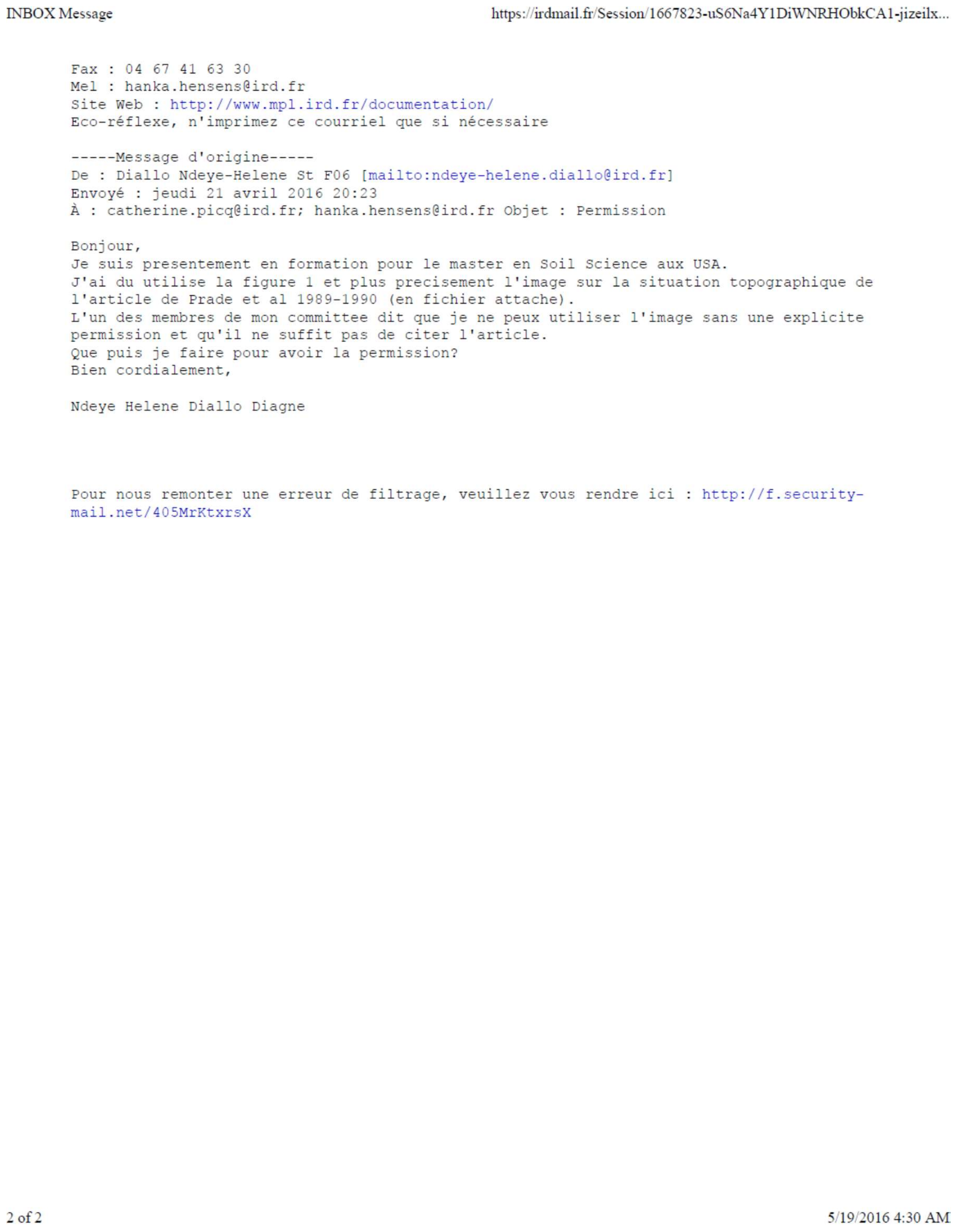

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Appendix B. Selected physical and chemical properties of the soil at Djibelor site.

Horizons	Depth (cm)	pH1:1	Tons CaCO3/1000 tons material	% Organic Matter	Organic C mg/g	P mg/l	K mg/l	Total dry Sulfur %	Al_{KCl} mg/l	Fe_{M1} mg/l	EC dS/m	Particle size %			
												% Sand	% Silt	%Clay	Texture
Apz1	0-13	3.36	8.88	2.7	13.7	12	38	0.40	86.3	146.7	56.75	58.5	26.8	14.7	SL
Apz2	13-22	3.36	2.43	1.8	10.5	5	19	0.06	42.0	82	14.26	62.4	20.7	16.8	SL
BAg	22-43	3.58	7.54	1.5	9.1	10	14	0.04	54.7	14.8	7.525	54.8	24.6	20.6	SCL
Btg1	43-58	3.29	9.74	0.4	2.8	5	12	0.02	59.1	6.5	2.755	55.5	21.1	23.5	SCL
2Btg2	58-72	2.91	4.77	0.3	1.0	1	28	0.01	91.6	29.8	4.42	71.0	5.5	23.5	SCL
2Btg3	72-90	2.72	2.9	0.5	1.9	1	29	0.03	100.7	20.3	4.79	62.2	3.7	34.1	SCL
2BCtg	90-110	2.62	2.67	0.7	2.7	1	19	0.03	65.5	48.6	5.755	71.3	2.6	26.0	SCL
2Cjz	110-132	2.5	12.95	0.6	2.3	4	2	0.39	91.5	1457.8	12.85	87.9	2.7	9.4	LS
2Cjvz	132-150	2.45	22.72	1.7	3.6	3	2	0.44	65.9	650.1	9.355	84.9	4.3	10.7	LS