



Mini Review

Response of Soil Proteobacteria to Biochar Amendment in Sustainable Agriculture- A mini review

Hua Zhang^{1†}, Farman Ullah^{2†}, Rafiq Ahmad³, Sayed Usman Ali Shah⁴, Abdullah Khan⁵, Muhammad Adnan^{5*}

¹Guangxi Key Laboratory of Rice Genetics and Breeding, Nanning, 530007, China.

²Department of Forest Resources Management, Nanjing Forestry University China

³Institute of Soil and Water Conservation, Northwest A& F University, Yangling, China.

⁴College of Life Science and Technology, Guangxi University, Nanning China.

⁵College of Agriculture, Guangxi University, Nanning China.

Corresponding Author:
adnan.gxu786@gmail.com

[†]These authors contributed equally.

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ABSTRACT: In recent years, biochar application to soil has become more popularized due to its potential roles on soil fertility, plant growth, and development. In this review, we discussed the impact of biochar on the relative abundance of soil proteobacteria and its relationship with soil physiochemical properties under different rhizospheres. It was observed that biochar applied to different soil improved proteobacteria, and its lowest and highest relative abundance was ranged from 30-80%, respectively. A positive relationship of soil proteobacteria with soil pH, total nitrogen, available phosphorous, available potassium and total carbon were observed in several studies. Both the relative abundance of proteobacteria and its relationship with soil properties depend on biochar type, soil type, and fertilizers applied to the soil. Most of the ammonia-oxidizing bacteria including nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria belong to proteobacteria, which plays a significant role in nitrogen recycling that is beneficial for the plant growth, yield and fruits/seeds quality. Furthermore, a positive relationship between soil proteobacteria and plant yield was also highlighted. In this context, the use of biochar play a potential role to improve the relative abundance of proteobacteria in sustainable agriculture. We highlighted future research guidelines that might benefit the sustainable agricultural system. Moreover, further studies are needed to explore the potential role of biochar application on Proteobacteria families such as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria*.

KEYWORDS: Biochar, soil proteobacteria, soil properties, different rhizospheres, relationships

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1. Introduction

Soil microbes play a vital role in the ecological processes of soil containing organic matter decomposition and soil aggregates formation (Condon et al., 2010). This not only increases the soil fertility, but also enhance a series of ecosystems

(Lemanceau et al., 2015). Several studies have reported an increase in soil microbes diversity and biomass with the amendment of biochar to soil (Dangi et al., 2020; Diacono et al., 2011; Han et al., 2010; Meng et al., 2019). Meng et al. (2019) observed that wheat straw-derived biochar amendment to soil

significantly increased the abundance and diversity of plants beneficial bacteria in the rhizosphere of wheat seedlings. Among soil bacteria's, proteobacteria is the largest phylum of abundant bacteria, which are composed of mesophilic and neutrophilic bacteria (Fukuyama et al., 2010), and are related to a wide range of functions involved in carbon, nitrogen, and sulphur cycling (Mhete et al., 2020). Previous research studies have reported that nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria are a significant effects on the nitrogen cycle, and most ammonia-oxidizing bacteria belong to proteobacteria (Liang et al., 2012; Stein et al., 2003). Proteobacteria members are predominant in several soil ecosystems, including the rhizospheres, saline soil and semiarid soil (Mhete et al., 2020). Kersters et al. (2006) reported that proteobacteria consists of more than 460 genera and more than 1600 species, scattered over 5 major phylogenetic lines of descent known as the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria.

Improving the production of crops in modern agriculture increased the uses of fertilizer (Ali et al., 2020). Thus, the overuse of fertilizers can directly influence the growth of microbial populations as a whole by supplying nutrients and may affect the composition of individual microbial communities in the soil (Nakhro et al., 2010). Biochar (BC) is a carbon-rich, stable product that is produced by the burning of organic material (biomass) of agricultural and forestry wastes via a controlled process called

pyrolysis, and is well known for improving crop production and soil health (Ali et al., 2020; Ullah et al., 2021; Ali et al., 2022, Khan et al., 2021). Positive or negative effects of BC on total soil microbial community as well documented (Khan et al., 2014; McElligott et al., 2011; Nguyen et al., 2018). According to some reports, application of biochar to soil may provide a direct threat to soil flora and fauna, but it may also promote soil health, significantly change the make up of the soil biological community, and boost soil microbial biomass (Ullah et al., 2021; Liang et al., 2010; O'Neill et al., 2009; Jin, 2010). Meanwhile, due to the sensitivity of soil microbes, application of biochar to soil may have an impact on soil microbial populations, community structure, and physiological activities (Dempster et al., 2012; Dai et al., 2016; Lehmann et al., 2011). It has been noted that soil microbial abundance rose proportionally as biochar production rate improved (Gomez et al., 2014). As opposed to this, Ameloot et al. (2014) reported the opposite effects, claiming that 49 t biochar per ha introverted microbial activity and condensed both extractable phospholipids (PLFA) concentration and fungal abundance. Furthermore, (Ali et al., 2020; Han et al., 2017) reported that biochar addition to soil can considerably improve the soil pH, which potentially provides a more favorable habitat for microbial organisms, especially bacteria that are sensitive to pH (Han et al., 2017).

As a result, the application of biochar alteration in soil biological processes is a new field of study for soil science, and there are many anomalies that need to be explored. Recently many reviews have summarized the responses of soil biota and fertility to biochar

amendment (Lehmann et al., 2011; Ding et al., 2016), providing a good description of biochar application and soil microbial community composition. To date, no review is published that included detail of a single bacteria “proteobacteria” relative abundance in response to biochar amendment. Therefore, improvements in our understanding of biochar necessitate a review of its impacts on the abundance of soil proteobacteria and its relationship to soil properties to develop a road map for future research.

2. Changes in the relative abundance of proteobacteria in sustainable agriculture

Fertilizers are essential for better crop growth, yield and grain quality. Previously, it was reported that diverse fertilizers amendment can alter soil proteobacteria, for example, nitrogen application is increases for long term (Dai et al., 2018) or decreases (Kalivas et al., 2017) the relative abundance of proteobacteria. Another study analyzed the dominant microbiome at the phylum level and their results showed that the dominant phyla were proteobacteria ranging the relative abundance from 51 to 58% (Li et al., 2020). The negative impact of NPK fertilizer on soil proteobacteria were also reported by Soni et al. (2016) and Shao et al. (2018), and their results suggested that the relative abundance of specific bacterial phyla was influenced by soil chemical (salinity) and biochemical properties. Further, Liang et al. (2020) documented that the relative abundance of proteobacteria was significantly higher in bio-organic fertilizer treatments as compared to nitrogen fertilizer applied treatments (Table 1; Figure 1).

In contrast, Gu et al. (2020) concluded that nitrogen application increased the relative abundance of proteobacteria compared to control treatment. They highlighted that available nitrogen was the main factor, which may influence these organisms in the soil. However, 25 - 45% of the relative abundance of proteobacteria was found in grape rhizosphere treated with typical chemical fertilization for three consecutive years, which might due to containing more available N and available P than the rhizosphere soil of the other treatments (Wu et al., 2020). In the grassland rhizosphere treated with mushroom residue, the relative abundance of proteobacteria was recorded, ranging from 22-23% as compared to control treatment (Shang et al 2020). Their results are in agreement with previous studies on bacterial community composition in grassland soil (Chen et al., 2017; Xu et al., 2018; Nacke et al., 2011). Sun et al. (2019) reported that proteobacteria relative abundance ranges about 58% in grassland with nitrogen fertilizer application.

Furthermore, the classes of proteobacteria, including *Alphaproteobacteria* and gamma proteobacteria, were increased in 269 kg N ha⁻¹ compared to the control treatment (Sun et al., 2019). *Betaproteobacteria* and *Deltaproteobacteria* decrease and were not significantly affected by nitrogen fertilizer (Sun et al., 2019). Li et al. (2021) reported that nitrogen fertilizer under different irrigation levels improves substantially the abundance of proteobacteria followed by Bacteroidetes Actinobacteria, Acidobacteria. The range of proteobacteria abundance was recorded from 28.46–37.78% t 120 kg N ha⁻¹ in the wheat field (Li et al., 2021).

Table 1. The relative abundance of soil proteobacteria in different rhizosphere and different fertilizers applications.

Rhizosphere	Proteobacteria relative abundance	Fertilizer used	References
Maize	51.17–57.89%	NKP	Li et al. (2020)
Wheat	29.67%–34.15%	Bio-organic fertilizer	Liang et al. (2020)
Sugarcane	31.23–40.68%	N375 and N563 kg ha ⁻¹	Gu et al. (2021)
Grape	25–35%	NPK fertilization for three consecutive years	Wu et al. (2020)
Hulunbuir Grassland Ecosystem	22–23%	Organic fertilizer	Shang et al. (2020)
Grass land	58%	Nitrogen Fertilizer	Sun et al. (2019)
Sugarcane	45%	NPK	Khan et al. (2021)
Tomato	86–89%	Chicken manure	Haq et al. (2021)
Wheat	28.46–37.78%	N fertilizers	Li et al. (2021)
Maize	23.2%	N fertilizer	Muhammad et al. (2022)
Sugarcane	47%	NPK	Khan et al. (2022)
Rice	30–40%	Manure + NPK	Iqbal et al. (2022)

In addition, a recent study reported that N fertilizer at the rate of 300 kg ha⁻¹ under different irrigation levels increased proteobacteria and was the most abundant bacteria, ranging by 23%, followed by Firmicutes (Muhammad et al., 2022). Similarly, Khan et al. (2022) reported that nearly 47% of the total species found in the rhizosphere of ratoon crops were proteobacteria, nevertheless, the fraction of several bacterial species changed during the second rationing.

Several studies reported the relationships among soil properties with environmental factors and proteobacteria. The positive relationship of proteobacteria with soil P, N-NH₄, NO₃-NO₂-N, Nt, Ct, OM, and moisture was recorded in grassland soil treated with

NPK fertilizers (Pan et al., 2014). Khan et al. (2021) evaluated the response of bacteria at the phylum level in the sugarcane rhizosphere, and they observed 45% of relative abundance of proteobacteria at phylum level, whereas at class level Gamma-proteobacteria were the highest by 30% of relative abundance. Meanwhile, proteobacteria in soil were found positively correlated to the metal nutrients availability, especially to calcium (16 OTUs) and magnesium (19 OTUs) (Zhang et al., 2020). Haq et al. (2021) determined that the relative abundance of proteobacteria was higher in the soil treated with chicken manure ranging from 86–89% in the tomato rhizosphere as compared to control.

The changes in soil proteobacteria are mostly attributed to the status of nutrients available in the soil. Likewise, numerous studies observed that the abundance of soil proteobacteria was significantly influenced by soil physiochemical properties (Li et al., 2020; Liang et al., 2020; Gu et al., 2021; Wu et al., 2020; Shang et al., 2020; Sun et al., 2019; Khan et al., 2021). Currently, improving soil physiochemical properties and biochemical properties facing a great challenge, around the globe agronomist suggested the use of biochar in sustainable agriculture to improve soil health and crop production (Ali et al., 2020; Ullah et al., 2021; Ali et al., 2021; Khan et al., 2021; Ding et al., 2016; Diatta et al., 2016; Imran, 2021). Numerous studies focused as whole soil microbe's responses to biochar in different rhizosphere, we reviewed the article on the plant growth promoting bacteria "proteobacteria" in response to biochar to understand the role of biochar in relation to soil most abundant bacteria.

3. Biochar and soil proteobacteria

The use of biochar as a targeted method for managing soil biota is gaining popularity; while in advertent changes in soil biota as a result of biochar use are also strong concerns. This is an essential area of research because the health and diversity of soil bacterial populations are important for soil function and ecosystem services, which in turn affect soil structure and stability, nutrient cycling, aeration, water use efficiency etc. (Table 2) (Khan et al., 2021). Therefore, we assumed to review the relative abundance of soil proteobacteria under different biochar

amendment in different rhizospheres. Results showed that soil proteobacteria was found the most abundant bacterium in response to biochar treatments as shown in figure 1. Moreover, Yin et al. (2021) reported that compared to control, corn stalk biochar application increased the relative abundance of proteobacteria by 13% in rice rhizosphere under pot experiment. This is because proteobacteria is a eutrophic bacterium, and biochar addition has been proven to improve the nutritional properties of albic soils (Fierer et al., 2007), resulting in an increase in proteobacteria abundance.

Fan et al. (2020) investigated that bacterial community composition after six years of biochar addition, and proteobacteria was the most abundant phyla accounting for 39.0–40.4% of the total composition. However, in their study biochar did not change the abundance of proteobacteria compared to control, while the relative abundance of Nitrospirae and Verrucomicrobia phylum increased but that of Acidobacteria phylum decreased significantly in biochar amended soils. Similarly, Kong et al. (2020) recorded proteobacteria as most abundant phyla ranging from 48.26–82.32% in mountain soil treated with ground residue biochar. In contrast, Liao et al. (2019) documented that biochar addition to soil decreased the abundance of proteobacteria. Similarly, another study also found that the relative abundance of proteobacteria was much higher in the control treatment as compared to biochar treatments (Kolton et al., 2011). Besides, Zhang et al. (2020) reported that the relative abundance of proteobacteria was

Table 2. Response of proteobacteria to different biochar's in various rhizospheres.

Biochar type	Proteobacteria (Total relative abundance)	Rhizosphere	References
Rice straw and corn straw biochar	30.69-34.97%	Rice	Yin et al. (2021)
Corn straw at a pyrolysis temperature of 500 °C	39-40%	Rice	Fan et al. (2020)
Ground residue at a pyrolysis temperature of 500 °C	48.26–82.32%	Mountain	Kong et al. (2020)
Rice straw 500 °C,	35.45%	Tobacco fields	Zheng et al. (2021)
Cppl (Maluspumila Mill.) wood chip pyrolyzed at 500°C	43 and 35%	Intercropping of bean (Vicia faba L.), and a cereal crop, maize (Zea mays L.),	Liao et al. (2019)
Citrus wood	71 to 47%.	Pepper Plants	Kolton et al. (2011)
Maize straw (600°C)	73.54%±3.11	Sea grass	Zhang et al.(2020)
Cassava straw (400-500 °C)	30-50%	Rice	Ali et al. (2022)
Sewage sludge biochar (300-600 °C)	20-45%	Agriculture field soil	Ahmad et al. (2022)
Weed, Ageratina adenophora (Spreng.) (500%)	24-72%	Cucumber	Li et al. (2022)
Cotton straw biochar (450 °C)	90-96%	Cotton field Soil	Zhu et al.(2022a)
Rice Straw (500-600°C)	21%	Pomelo Orchard	Song et al.(2022)
Fruit tree residues (550 °C)	87.8–88.9%	Experimental Field (Clay soil)	Zhang et al. (2022b)
Rice straw (450 °C)	22.53%	Mountain Watershed	Wang et al. (2022)
Bark of Italian poplar (600°C)	30-50%	Polyethylene flowerpot	Zhu et al. (2022b)

found most abundant $73.54\% \pm 3.11$ for rhizosphere sediment under biochar application. In case of class level, on day 14 for sediment bacterial communities, *Alphaproteobacteria* with biochar addition treatments had a decreased in relative abundance, whereas *Gammaproteobacteria* had a higher relative abundance. (Zhang et al., 2020a). Zhang et al. (2022b) observed proteobacteria, Cyanobacteria, and Actinobacteria the most dominant phyla in clay treated with fruit tree residues biochar. Furthermore, they attributed that changes in microbial diversity was due to soil porosity, soil moisture content, organic matter N and P fertilizer (Zhang et al., 2022b).

After three years of biochar applied improved soil physiochemical properties which consequently improved soil proteobacteria accounted for 30-50% of total bacterial abundance under field condition (Ali et al., 2022). They observed that changes in bacterial abundance were strongly dependent on soil physiochemical properties. Recent research recorded that phyla proteobacteria was the most abundant bacteria and accounted for 40-50% of the total bacterial population in soil treated with biochar which can enhance the soil nutrient cycle (Ahmad et al., 2022). They observed that different pyrolysis temperature of biochar significantly affected soil bacterial community structure. However, an increase in soil enzymatic activities and a decrease in proteobacteria-relative abundance were recorded in soil treated with biochar applied compared to control and compost treated soil (Azeem et al., 2020). A higher range of 24 -72% of relative abundance of proteobacteria in cucumber under pot experiment condition was recorded by Li et al.

(2022). They further documented that the bulk soil bacterial populations did not change considerably or even reduced, the relative abundance of proteobacteria and Actinobacteria grew dramatically in the rhizobacterial communities. Similarly, 90-96% of proteobacteria relative abundance was recorded in soil treated with cotton biochar in ceramic pots (Zhu et al., 2022). They further observed that proteobacteria relative abundances was increased by 11.58% in biochar treatment.

4. Relationship of soil proteobacteria with soil properties under biochar application

Soil physiochemical properties play a vital role in soil microbial abundance including soil proteobacteria. While, biochar is a key factor of changing soil physical and chemical properties (Ali et al., 2020; Ullah et al., 2021; Ali et al., 2021). An earlier study found that the first axis of soil microbial community composition is considerably impacted, either favorably or negatively, by soil pH, TN, C/N, TC, AK, TK, and AN (Fan et al., 2020).

Fan et al. (2020) reported that the abundance of proteobacteria was significantly positive associated with soil pH, TC, TN AK and AP, while it's was negatively correlated with TK and AN under biochar application. Which indicates that biochar application speckled bacterial community composition indirectly through variation in soil properties. A negative correlation between soil pH, MBN and MBC with soil proteobacteria was observed by Kong et al. (2021). However, under NPK fertilization, Khan et al. (2021) reported a positive correlation of soil proteobacteria with soil N, P and K in sugarcane rhizosphere along with different

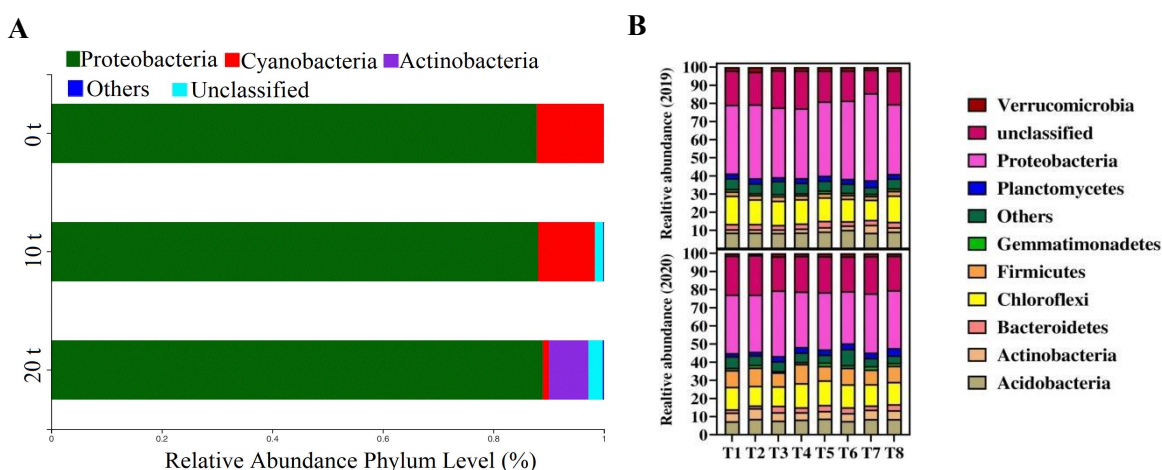


Figure 1. Relative abundance of dominant bacterium with biochar addition over 45 days incubation applied treatments (A) (Sun et al., 2021) and after two years in rice field (B) (Ali et al., 2022).

cultivars in Guangxi Province of China. A positive relationship of relative abundance of proteobacteria with soil pH, moisture, AP, and AK were recorded under different nitrogen and irrigation levels (Li et al., 2021). However, a negative relationship with soil pH, and positive relationship with soil enzymatic activities with bacterial phyla under N and irrigation levels was reported by Muhammad et al. (2022). According to Ali et al. (2022), proteobacteria ($R=0.32$) have a substantial positive correlation with paddy rice grain yield, whereas Chloroflexi, Firmicutes, Gemmatimonadetes, and Verrucomicrobia have no visible relation with rice grain yield. Furthermore, another finding revealed a close correlation between the relative abundances of Acido-bacteria and proteobacteria and the soil biochemical characteristics (pH, C/N ratio, and soil enzyme activity), suggesting that the soil biochemical characteristics had a significant impact on the relative abundances

of Acidobacteria and proteobacteria (Zhu et al., 2022).

5. Mechanism of biochar to improve soil proteobacteria

Biochar application is well-known to improve soil health, including enzymatic activities, physiochemical properties, microbial biomass carbon and nitrogen. These factors are directly or indirectly correlated with soil bacterial abundance and composition structure and proteobacteria accounted in the top abundant bacteria's. Based on these findings, a hypothetical mechanism of biochar was proposed affecting soil proteobacteria abundance. Firstly, biochar application could consistently improve soil pH (Ali et al., 2020; Ullah et al., 2020), total nitrogen (Son et al., 2022; Ullah et al., 2021a), available phosphorous (Tesfaye et al., 2021; Ullah et al., 2021b), available potassium (Wang et al., 2018), total carbon (Ali et al., 2021).

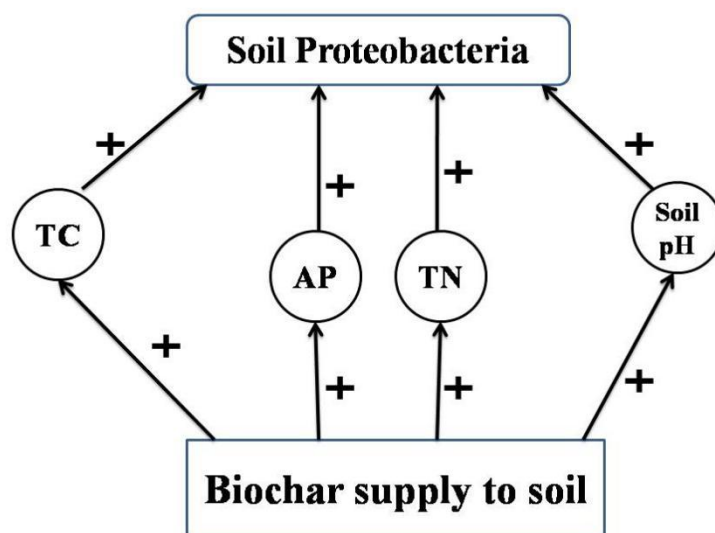


Figure 2. A Mechanism involving the soil properties has an influence on soil proteobacteria abundance under biochar. (+) and (-) represented increased and decreased effects, respectively. Note; TC-total carbon, AP-available phosphorous, TN-total nitrogen,

Secondly, these soil indicators were significantly positively correlated with soil bacterial abundance and especially proteobacteria (Ali et al., 2022; Song et al., 2022; Khan et al., 2022). In other words, the model has highlighted that the soil physiochemical properties could positively affect soil proteobacteria under biochar applied treatments. In addition, since biochar could distinctively alter soil proteobacteria, it remains interesting to explore whether it also affects Proteobacteria families such as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria*.

6. Conclusion, limitations and future aspects

The available literature provides ample justification for further investigation into the effects of biochar on proteobacteria, which

contributes to soil fertility indirectly through ammonia-oxidizing bacteria. The abundance of this bacterium significantly influences plant roots, growth and yield. A great abundance of proteobacteria in soil with biochar application is relatively well established (Table 2; Figure 2). Apart from using biochar as inoculants carriers, little information about using biochar to manage proteobacteria on class, order, family, genus and species level. The knowledge gap needed an urgent attention including biochar effects on ammonia-oxidizing bacteria including nitrogen-fixing bacteria, ammonia-oxidizing bacteria, cellulose-decomposing bacteria, nitrifying bacteria and denitrifying bacteria belongs which belongs to proteobacteria. Furthermore, the effect of biochar under different rhizosphere on proteobacteria family such as *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*,

acteria and *Epsilonproteobacteria* need to be documented. Important questions emerged from a biochar-sphere perspective: How far does the influence of biochar reach into different soil *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Epsilonproteobacteria* abundances? What are the critical soil physiochemical properties influences proteobacteria species?

Authors Contributions:

Z. H and M.A conceived the main idea of research, Z.H and F.U., wrote the review article. S.U.A.S., R.A, M.A, F.U., A.K., and Q.H revised the draft and provided suggestions. All authors have read and agreed to the published version of the review article.

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