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Review

The Effect of Different Biochar Characteristics on Soil Nitrogen Transformation Processes: A Review

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Abstract: For the last 30 years, interest has focused on biochar and its potential to store carbon in soil to mitigate climate change whilst improving soil properties for increased crop production and, therefore, could play a critical role in both agricultural sustainability and broader environmental aims. Biochar, a carbonaceous product, is formed from organic feedstock pyrolysised in the absence of air and, therefore, is a potential means of recycling organic waste. However, different feedstock and pyrolysis conditions result in a biochar with a range of altered characteristics. These characteristics influence nitrogen transformation processes in soil and result in the metabolism of different substrates and the formation of different products, which have different effects on agricultural yield. This paper reviews how the production of biochar, from varying feedstock and pyrolysis conditions, results in different biochar characteristics that influence each stage of the nitrogen cycle, namely processes involved in fixation, assimilation, mineralisation and denitrification. The nitrogen cycle is briefly outlined, providing a structure for the following discussion on influential biochar characteristics including carbon composition (whether recalcitrant or rapidly metabolisable), mineral composition, surface area, porosity, cation exchange capacity, inhibitory substances and pH and so on. Hence, after the addition of biochar to soil, microbial biomass and diversity, soil porosity, bulk density, water-holding capacity, cation exchange capacity, pH and other parameters change, but that change is subject to the type and amount of biochar. Hence, products from soil-based nitrogen transformation processes, which may be beneficial for plant growth, are highly dependent on biochar characteristics. The paper concludes with a diagrammatic summation of the influence of biochar on each phase of the nitrogen cycle, which, it is hoped, will serve as a reference for both students and biochar practitioners.

Keywords: biochar; nitrogen fixation; mineralisation; denitrification; soil amendment

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1. Introduction

Every year, 2 billion tonnes of solid waste are generated globally [1], 30 billion tonnes of carbon dioxide (CO_2) [2] are emitted and 12 million hectares of agricultural land are lost to soil degradation, leading to a potential loss of 20 million tonnes of grain per annum [3]. This, as well as an ever-growing food and energy requirement as tastes shift towards a more technologically driven lifestyle, means that further stress is placed on Earth's already depleted and increasingly polluted resources [4,5]. Hence, there is now a focus on technologies that can reuse waste, reduce emissions, mitigate climate change, generate energy and support food production. One such technology is pyrolysis and the production of biochar. Pyrolysis generally refers to a reaction in an inert environment [6]. In this case, it is the thermal degradation of organic material (wood, waste or energy crops) in the absence of oxygen [7]. As well as the production of energy, the co-product of pyrolysis is a carbonaceous product referred to as biochar.

Since 1993, interest in biochar has focused on whether it can be used to store carbon in soil whilst improving soil properties for improved crop production [8,9]. Carbon storage in soils is seen as a means to mitigate greenhouse gas (GHG) emissions through the sequestration of CO_2. Woolf et al. [10] analysed what level of carbon sequestration, through the production of dedicated biomass for biochar, might be practical to mitigate climate change, without compromising food security, habitat or soil conservation. They estimated that a maximum of 1.8 Pg CO_2-C equivalent (CO_2-Ce) per year (or 12% of current anthropogenic emissions) might be achievable, although estimates range from 0.7 to 1.8 Gt CO_2 eq y^{-1} [11].

Biochar improves the fertility of some soils through the direct provision of nutrients such as nitrogen, phosphorus and potassium. Table 1 provides a selected range of biochar nutrient levels. Biochar can also have a liming effect, influencing pH such that nutrients become more available [12,13]. Most biochars have a high pH (when compared to soil), which results from their ash content and base cations, but also due to intrinsic alkaline organic functional groups [14]. However, these values are governed by both feedstock and pyrolysis conditions. For instance, one study found that the pH of herbaceous biochars was two units higher (9.4) than woody biochars (7.4) due to higher concentrations of ash [15]. pH was also found to be higher in biochars from leguminous feedstock (9.02 to 10.35) than in non-leguminous feedstock (8.00 to 9.24) [16]. Here, carbonates and organic anions of carboxyl and phenolic groups were the main alkalis but, again, this varied with feedstock. The influence of biochar on soil pH is key because it has been shown to improve gross mineralisation rates, immobilisation rates and heterotrophic nitrification and have an overall positive effect on soil nitrogen retention [17].

Table 1. Selected physical and chemical parameters for a range of biochars.

Biochar Feedstock	C g kg^{-1}	N g kg^{-1}	C:N Ratio	P g kg^{-1}	K g kg^{-1}	pH	Porosity Surface Area M^2 g^{-1}	Reference
Wood chips	708	10.9	65	6.8	0.9		4.82	[16]
Wood chips	720	10.8	67	1.3		9		[17]
Green wastes	680	1.7	400	0.2	1.0		2.10	[18]
Poultry litter	380	19	19	25.2	22.1			[18]
Cow manure		20		280	20	10		[19]
Maize cobs	568	0.8	710		18.7	11		[20]
Maize straw	489	12.5	39			10	4	[21]
Pig manure	511	21.1	24	38.5		10		[17]
Swine manure	422	4		30	8	10		[22]
Birds foot trefoil foliage	600	32.2	18			9		[23]

Hence, these differences in biochar qualities result in different effects on crop yield. For instance, corncob biochar on infertile acidic soil reduced maize yield, which may have resulted from the biochar's high volatile matter content and bioavailable carbon. This labile carbon fuelled an increase in micro-organism growth, drawing nutrients from the soil and resulting in nitrogen immobilisation [18]. The availability of nitrogen for crop growth is a key agronomic parameter. Nitrogen is an essential plant nutrient and a key element in the development of organic molecules including amino acids, amino sugars, proteins, deoxynucleic acid (DNA), chlorophyll, etc. Nitrogen is very reactive and therefore has a very complex cycle through the soil system, sometimes transforming quickly between inorganic forms as gasses such as NH_3, N_2, N_2O, and NO, in ionic form in soil, NH_4^+, NO_2^- and NO_3^-, and organic forms. Soils vary considerably in their total nitrogen concentrations from around 0.06% to 0.30%, and more than 90% of this is in organic form [19].

Biochar has been reported to reduce nitrogen leaching losses [20]. However, these ameliorative effects can vary depending on the carbon content of the amended soil, the biochar's carbon content, soil textural differences, soil water-holding capacity and the soil micro-organism community amongst other parameters [21–25]. Indeed, in their global meta-analysis of the effect of biochar on yield in temperate and tropical systems, Jeffery et al. [26] found that, on average, there was no beneficial effect on crop yields in temperate latitudes, but there was an average 25% increase in yield in the tropics. This was because in low-pH, low-fertility soils, the liming and fertilisation effect of biochar was enough to increase yield [26].

However, biochar's influence on the native micro-organism community can exacerbate nitrogen losses from soil systems, depleting nitrogen from soil but also increasing pollutant release. For example, whilst several studies have reported that the incorporation of biochar into different soils reduced N_2O emissions [27–31], other studies have reported that biochar incorporation results in an increase in N_2O emissions [32,33]. However, the magnitude of change in N_2O emission because of biochar incorporation is dependent upon experimental conditions, biochar type, application rate, soil properties, and chemical forms of added fertilizer [30]. Nevertheless, as N_2O has a global warming potential of 298 times that of CO_2 [34], research on techniques to minimise emissions is obviously critical.

It would appear then that biochar could prove to be a sustainable amendment for agricultural use, capable of both GHG emission reduction and soil enhancement. However, as noted above by Woolf et al. [10], it is critical that its feedstock comes from sustainable sources and does not compromise a nation's ability to grow its own food and its food security by growing feedstock on productive land [11]. In addition, this review considers how the application of biochar to soil may influence nitrogen transformation in terms of when and where soil nitrogen is partitioned. This is a key sustainability issue because it either exacerbates or mitigates the effect and level of nitrogenous inputs and outputs to and from the soil system acting as either pollutants (for instance, in the case of GHG emission) or sources of agronomically beneficial nitrogen.

2. Phases of the Nitrogen Cycle

Here, we briefly outline the nitrogen cycle and components upon which biochar may act. This forms the basis for the following summary of recent and older investigations on the influence of different biochar characteristics on nitrogen transformations in soil. Both agronomically beneficial and environmentally damaging issues are discussed. Finally, the findings are restructured so that all influential parameters at each stage of the nitrogen cycle, here defined as fixation (of atmospheric N_2), assimilation, mineralisation and denitrification, are elucidated. We hope this review will provide a reference for students, researchers and soil remediation practitioners on the influence of biochar on soil nitrogen transformation processes.

The soil system is open to the atmosphere and nitrogen cycles between them. The first input of nitrogen from the atmosphere is either through lightening, deposition or biological nitrogen fixation (BNF) (Figure 1). This is an important phase of the nitrogen cycle, capturing N_2 and transforming it to NH_4^+. This process fixes a considerable level of nitrogen year on year. For instance, prior to human intervention, BNF is estimated to have provided 58 Tg N yr^{-1} worldwide, and it still plays a critical role in maintaining soil fertility [35]. It uses the soil's natural resources (native micro-organisms) and is, therefore, a free and potentially ecologically sound means of crop fertilisation.

BNF is carried out by several groups of prokaryotes, including *Azotobacter*, *Azospirillum*, *Rhizobium* and *Bradyrhizobium* [36]. These are either free living in the soil or form symbiotic relationships with plants (around or on the root surface) and both utilise enzymatic processes to catalyse the conversion of N_2 into NH_3. This conversion to ammonia (or ammonification) is then followed by a process of nitrification to nitrites and nitrates. This process is undertaken by both heterotrophic and autotrophic micro-organisms with the bacterium, such as *Nitrosomonas*, converting NH_3 into NO_2^-, and *Nitrobacter* converting NO_2^- to NO_3^- [36]. It is in these forms that plants and micro-organisms can assimilate NH_3 and NO_3^- into their structures and the nitrogen then exists in organic form. Upon their death, or through excreta, these forms are then mineralised back again into inorganic form.

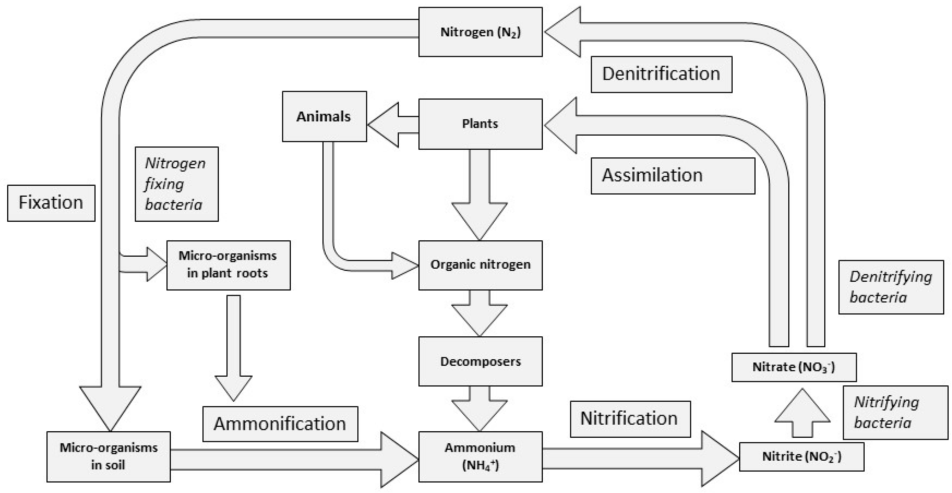

Figure 1. Simplified nitrogen cycle.

They are mineralised by micro-organisms that derive energy from the oxidation of organic nitrogen to NH_4^+. This is then available to be assimilated and incorporated into amino acids or used for other metabolic purposes. If micro-organisms produce NH_4^+ in excess of their own requirements, the surplus is excreted into, in this case, the soil, and is available for assimilation by plants, or as the substrate for nitrification [37].

Finally, denitrification releases the N_2 originally acquired from the atmosphere by nitrogen-fixing bacteria. Again, it is a process facilitated by micro-organisms where NO_3^- is reduced through a series of nitrogen oxide products of decreasing oxidation states until molecular nitrogen (N_2) is all that remains. The process is of particular interest to climate change researchers as some of the intermediate products, nitric oxide and N_2O, are GHGs [38].

3. The Influence of Key Biochar Parameters on the Nitrogen Cycle

Biochar influences the composition, biomass and enzyme activity of micro-organism communities, including those with nitrogen-transforming capabilities. The mechanisms include the provision of recalcitrant and metabolisable carbon, mineral ions, its sorption phenomena, change in pH, as well as physical properties such as pore structure and surface area [7,15,39–41]. The change in these processes results in the production of more or less substrates and products in the nitrogen cycle.

3.1. The Influence of Recalcitrant and Metabolisable Carbon

Critical to biochar's ability to store carbon in soil are its aromatic rings, which are highly stable. Aromatic rings include six carbon structures such as benzene, which have strong chemical bonds very resistant to attack by micro-organisms [42]. It is this resistance that confers recalcitrance to biochar stored in soil and, hence, its role as a climate change mitigation tool. However, biochar contains other carbon compounds, such as aliphatic carbon chains. These structures have weaker chemical bonds easily broken by micro-organism enzymes and are thus used as an energy source for respiration and therefore result in the ultimate release of CO_2. Hence, although biochar has a carbon content that can range from 50 to 95%, some of this is metabolically available and will therefore influence micro-organism growth in the short term [43,44]. The amount of metabolically available carbon depends on the biochar feedstock and pyrolysis condition. For instance, where the production of biochar involves a shorter duration in the reactor, there may be an incomplete conversion of the feedstock biomass to more recalcitrant forms, leaving a greater fraction of unconverted cellulosic and hemicellulosic forms [45]. Any carbohydrates that remain after the pyrolysis process are rapidly metabolized by soil micro-organisms and support the growth of heterotrophic populations creating changes in the relative abundance of different families [39,46,47]. However, the consequence of this growth and change in abundance of different micro-organism populations varies with the phase of the nitrogen cycle. For instance, biochar addition creates a change in abundance of Bradyrhizobiaceae and Hyphomicrobiaceae families with an increase of up to 11% relative abundance compared to control [39]. Yet, not all metabolically available carbon positively influences BNF rates. For instance, Güereña et al. [48] found that biochar with a reduced volatile matter component considerably increased micro-organism responses compared with unaltered biochar and, therefore, concluded that some volatile matter components were toxic to *Rhizobium*.

In general, the effect of biochar on soil micro-organism mass (and therefore soil respiration) is not well understood [49]. For example, some studies have found a 30% degradation of carbon in the first 30 years and very little thereafter (over a 100-year timeframe) [50], whereas others estimated a loss of 47% C within a 50-year timeframe [51]. Confounding factors include whether the response is dictated by the metabolisable fraction of the biochar or whether the native micro-organisms have the necessary enzymes to mineralise that available carbon [41,46].

There are potentially negative agronomic consequences resulting from the provision of metabolisable carbon. Although biochar may improve nutrient assimilation by micro-organisms, there may be a consequent decrease in assimilation by crop plants. For instance, biochars with a high aliphatic concentration are more easily degraded by soil organisms, causing an increase in their growth rates and consequent short-term assimilation and immobilisation of nitrogen, impeding supply to crop plants and reducing yield [18,52].

Conversely, biochar also stimulates the growth of micro-organisms able to mineralise recalcitrant nitrogen found in soil organic matter [53]. For instance, one N^{15} isotope tracing study using maize biochar found that nitrogen mineralised to NH_4^+ came from soil organic matter, which was otherwise generally resistant to micro-organism attack [28]. They hypothesised that this was due mainly to improved metabolisable carbon availability supplied by biochar pyrolysised at a low temperature. The subsequent elevated growth in micro-organism population could not be met with available nitrogen sources in the soil resulting in the mineralisation of recalcitrant soil organic matter [28,39,54]. Yet other studies have found no effect on gross mineralisation [55,56]. It is likely that these divergent effects are dependent on two main attributes. Firstly, the C:N ratio of the added biochar, and secondly, the nature of the native soil micro-organism community and its genetic capability to mineralise recalcitrant nitrogen. Contrasting results can be obtained if soils have different levels of nitrogen availability because soil organisms will only mineralise recalcitrant nitrogen if readily available nitrogen is low. On the other hand, in nitrogen-rich biochars, such as those from manure and maize, the excess nitrogen allows mineralisation rates to increase [28,57].

A key phase within nitrogen transformations is denitrification, because, if incomplete, it can lead to the emission of N_2O. In their work with wood and poultry manure biochars, Singh et al. [22] found that, generally, biochar-amended soils produced significantly lower N_2O emissions than their respective controls but its efficacy in this regard was soil-dependent. They subjected two contrasting soils with different biochar treatment rates to wet–dry cycles. By the third cycle, they found that all biochar treatments consistently decreased N_2O emissions, cumulatively by 14 to 73% from the Alfisol and by 23 to 52% from the Vertisol, relative to their controls. However, some biochars produce higher N_2O emissions [22,30,58]. For example, a poultry manure biochar produced at 400 °C had the highest metabolisable nitrogen content. It appears that high metabolisable carbon and nitrogen combine to promote the activity of soil denitrifiers and therefore high N_2O emission [22]. Hence, where biochar provides a high metabolisable carbon content, either with or in the presence of a high nitrogen content, this can result in increased N_2O emission. In this study, it was also found that N_2O and CO_2 emission were initially positively related, and this was consistent with other studies (for example, [59]), but that this relationship disassociates due to a rapid increase in micro-organism growth supported by the provision of metabolisable carbon after two or three days. Furthermore, the addition of biochar with metabolically available carbon leads to temporary anaerobic conditions and the enhancement of N_2O-reductase resulting in a decrease in N_2O release (and an increase in N_2) [22,59,60]. This suggests that the initial peak of N_2O release is short-lived [61]. In their review of the potential for biochar as an abatement technology for GHG, [62] also found contradictory results with much depending on biochar type.

Biochar influences the cycles of carbon already in the soil, most notably organic matter and humus. Organic matter plays a vital role in the functioning of a healthy soil. Derived from the biomass of both plants and animals (for example, roots and manure), organic matter has a high CEC, helps retain water in the soil and provides soil structure. Organic matter decays over time, releasing its nutrients. When the level of decay slows significantly or stops, and the organic matter becomes stable, it is referred to as humus. Humic acids comprise a large group of chemicals, which perform a vital role in soil health contributing to soil moisture and nutrient retention, as well as the bulk density of soil [63]. There is now some evidence to suggest that biochar serves to protect humic acids from decomposition [64]. It is thought that this may be due to a physical 'shielding' by biochar of humic acids as micro-organisms cannot access the smallest biochar pores [41].

In some studies, biochar promoted the degradation of organic matter, for instance, with newly incorporated plant residues. Using ^{14}C-labeled maize residue, Awad [65] reported that biochar in both sandy and sandy loam soils stimulated soil micro-organisms, causing a significant increase in the presence of extracellular enzyme activities and consequently faster plant residue decomposition. The decomposition of plant residue was more pronounced in sandy soil, where it accounted for 23% of ^{14}C input, whereas in sandy loam soil, increased plant residue decomposition did not exceed 14% compared to untreated soils [65]. This may have been due to differences in the nature and quantity of pre-existing soil organic matter in both soils.

3.2. The Influence of Mineral Ions, Provision, Availability and Uptake

Biochar offers the opportunity to improve soil fertility through the direct provision of mineral ions. The quantity and type of minerals depend on pyrolysis conditions and feedstock. Hardwood biochars tend to have lower mineral contents (5–10%), whereas chicken litter waste can have an ash content of up to 64% [66]. The presence of mineral ions can have a significant effect on fixation rates. For instance, Rondon et al. [31] found that biochar significantly enhanced biological fixation by *Phaseolus vulgaris* and accounted for the increase in fixation rate through the increased availability of molybdenum and boron provided by biochar. They noted that the improvement in BNF, as well as the increase in biomass, of *Phaseolus vulgaris* (5–39%) was above those normally provided by recommended commercial fertiliser applications. Similarly, in *Glycine max* L., the enhancement of nodulation and BNF response to carbonized organic materials was due to an increase in available sulphur [67].

Even low-nutrient biochar has the potential to elevate nutrient availability. In their greenhouse experiment with seven different biochars, [48] stripped the biochars of mineral or volatile matter, or both, and left some untreated. The untreated biochar soil treatment planted with *Phaseolus vulgaris* resulted in an increase of 2126% in nitrogen fixation over the control average (as well as a 262% increase in shoot biomass, a 164% increase in root biomass and a 3575% increase in nodule biomass). The stripped biochar revealed that simple mineral nutrients provided by the biochar were only slightly responsible for these increases. For instance, although the amount of nitrogen fixed was significantly correlated with plant phosphorus uptake, it was *not* correlated with biochar phosphorus addition but rather improved phosphorus nutrition resulting from 360% greater mycorrhizal colonization with biochar additions.

Mycorrhizal root colonisation and hyphal responses to different biochars can vary substantially [68]. This may be due to different responses to a range of volatile matter compounds from contrasting biochars with mycorrhizal response being governed by carbohydrate availability or due to biochar providing physical protection from fungal grazers or the facilitation of root and hyphal exploration, facilitating improved access to nutrients for crop plants [48,68,69].

The provision of nutrients by biochar influences several different assimilation mechanisms. For instance, where biochar provides an increased cation concentration, plant water uptake increases due to the net increase in accumulated osmotically active ions such as potassium. This is key because it improves drought tolerance [70]. In addition, biochar appears to increase tap-root growth (and potentially fine root mass), which would increase water uptake from biochar pores [71]. Evidence for this improved water status with biochar has been demonstrated through lower proline (an amino acid associated with cell osmotic adjustment in leaves) concentrations and higher osmotic values in leaves, which may reflect an increased tolerance to drought conditions [72]. In addition, biochar may not only provide more nutrients but may reduce the leaching of nutrients from the soil, thereby maintaining availability [73]. One further mechanism is a biochar-mediated reduction in transpiration. For instance, one study found that biochar-amended plants produced larger leaves, and the plants also used slightly less water. This, together with increased leaf and root cell osmotic potential, may reduce sensitivity to drought stress and improve plant growth [70]. Another study showed that root growth was stimulated in the presence of 0.75% biochar. This again facilitated water uptake and soil nutrient acquisition and, therefore, exerted beneficial effects on photosynthesis and lowered oxidative stress [74].

3.3. The Influence of Porosity on Soil Structure, Water and Gas Dynamics

Due to its porous nature, biochar can significantly increase gas transport in soil as well as a soil's water-holding capacity (WHC) [7,75–77]. This is because pyrolysis results in an interconnected network of micropores, mesopores and macropores [41]. The distribution of pore sizes is governed by feedstock, with wood feedstock developing larger pores [75], and by pyrolysis conditions, with those biochars produced at high temperatures via a slow process more likely to produce more macropores (that is, greater than 50 μm in diameter) [75,78]. In addition, as biochar has approximately half the tensile strength of soil, it can reduce overall soil tensile strength, therefore reducing soil mechanical impedance [79], improving root elongation as well as mycorrhizal proliferation, thereby improving plant access to and assimilation of nutrients [7].

Biochar has been reported to improve crop yield through its effect on soil structure [80]. In some cases, biochar addition increased aggregate stability and reduced the detachment of colloidal material, improving soil structure [81]. However, in coarser soils, there was no such enhancement [82]. Where improvements were found, this may have been due to mechanisms such as carboxylic and phenolic functional groups on aged biochar surfaces, which form attachments with soil mineral surfaces. Also, a high CEC allows for cation bridge formation contributing to structural stabilisation [52].

Differences in biochar porosity resulting from different feedstocks have a direct influence on micro-organism population. This is because the adhesion of bacteria to biochar may be influenced by pore size [83]. *Bacillus mucilaginosus* and *Acinetobacter* sp. need a pore size of 2–4 μm if they are to enter [84]. In pores, they are better protected from dehydration and grazers and competitors. Surface tension holds water in the biochar, but it does so preferentially, with smaller pores exerting a greater holding capacity than larger ones. Equally, any increase in overall pore volume can increase water-holding capacity and provide greater resistance to water loss in drought-prone areas [85]. This balance is essential for nitrification, the optimum condition for which is 60% water-filled pore space (WFPS) [86]. The mode of application can have a critical influence, however. For instance, dry biochar is hydrophobic and may cause hydrophobicity in soil, interrupting water infiltration [87].

The effect of biochar on WFPS and soil aeration is often cited as the means through which complete denitrification is promoted and N_2O emission reduced [21,88]. Denitrifiers are highly sensitive, requiring an oxygen concentration of less than 10% to denitrify [89].

The porous nature of biochar provides shelter, water and oxygen resulting in the rapid growth of heterotrophs and, therefore, the depletion of oxygen and the creation of anaerobic microsites [88]. Oxygen partial pressure strongly influences both denitrification and nitrification rates [89] and therefore any anaerobic microsites that may form around biochar particles may elevate N_2O reduction activity [88].

Longer-term studies revealed the influence of different processes. For instance, in a seven-month study, biochar did not promote the reduction in N_2O to N_2, rather, the most prominent biochar-induced reduction in N_2O resulted from an increase in metabolisable carbon [27]. Equally, in their work with fifteen different soil types, Cayuela et al. [90] found that the mechanism for reduction in N_2O to N_2 was not linked to an increase in soil aeration but was closely related to soil texture with fine soils promoting the last step of denitrification. Equally, where biochar would not significantly influence WFPS, they found that the effect of biochar on N_2O production from denitrification did not correlate with the increased C:N ratio supplied by the biochar. Hence, micro-organism immobilisation of NO_3^- was not a driving mechanism for the observed N_2O reductions [90]. Neither, it appears, was nitrite, which can have an inhibitory effect on the action of N_2O reductase [91], reducing the production of the final product, N_2. Hence, biochar, in reducing the release of a GHG, would be an appropriate amendment for agroecological systems, but again, the choice of biochar type, given the soil type, would need to be carefully considered if any increase in N_2O was to be avoided or minimised.

3.4. The Sorption of Mineral Ions, Signalling Compounds, Heavy Metals and Organic Pollutants

Some biochars can be effective in adsorbing NH_4^+ and NO_3^- from the soil [92]. This apparent disadvantage, however, may lead to an increase in the ability of the soil ecosystem to feed plants as this reduced nitrogen availability to plant roots stimulates increased nodulation in legumes [64,93]. Root nodulation can influence the rate of biological fixation, and both the nodulation rate and development, as well as nitrogenase activity, can be affected by the presence of biochar. For instance, pyrolysised bamboo increased root nodulation by 243% and resulted in increased soybean growth [94]. However, another study found that three years after biochar application, regardless of the application rate, there was no significant difference in the total number of root nodules in clover between control and biochar-amended soil, although the level of nitrogenase activity in individual nodules was significantly higher in the biochar-treated soil [95].

Even though biochar can influence the structure of a micro-organism community to promote one nitrogen process or another, its sorption powers can often confound the result. In an experiment comparing biochar alone and biochar that had been shaken with dairy effluent for 24 h, both biochar treatments reduced net ammonification by 220% compared with soil alone. This suggested that the rate of nitrification was higher than the rate of ammonification. However, it appears that these rates were not changed in response to an increase in nitrifiers because CO_2 emissions did not rise. Hence, it was postulated that the reduction in NH_4^+ was more likely due to its adsorption to biochar rather than immobilisation [96]. Similarly, ammonification was enhanced when a metabolisable organic nitrogen substrate was added to forest soil after fire, suggesting that the process is substrate-limited [97].

Again, biochar may influence the denitrification process by limiting micro-organism access to substrates. For instance, in one study, an acidic biochar absorbed NH_4^+, not only reducing the NH_4^+ leaching rate but also decreasing the NH_3 volatilisation rate due to a reduction in substrate for the denitrification process [98]. However, the decrease in volatilisation rate was driven mainly by the acid–base reaction. Findings from another study concurred, concluding not only that the effectiveness of biochar to reduce N_2O emission was, in part, dependent on the sorption of NH_4^+, decreasing the overall availability of nitrogen to denitrifiers, but that this ability changed over time [22]. With ageing, the effectiveness of biochar to reduce N_2O emission (and NH_4^+ leaching) increased, as biochar surfaces become increasingly oxidised due to biotic and abiotic processes, potentially leading to an increase in cation exchange capacity [25], which may explain the reduction in available nitrogen [22]. Equally, biochar may also sorb N_2O directly, thus reducing emission; however, sorption sites are likely to be taken up by water, carbon dioxide, organic matter and other mineral ions and the competition for these sites has not been elucidated, requiring further investigation [99].

The mechanism of root nodule formation and BNF in leguminous plants requires infection by nodule-forming bacteria. This process is governed by chemotaxis involving signalling pathways, which are initiated by polyphenolic signalling compounds (for example, flavonoids) released by the host plant [40,100–104]. Biochar is highly effective at adsorbing signalling compounds so any incorporation of biochar into soil, certainly at higher rates, may interfere with these signalling pathways, potentially interrupting nodule development and therefore nitrogen fixation [40,101–104].

However, biochar's sorption capabilities may offer an advantage with regard to remediating contaminated soils [105–107]. Some organochlorine pesticides, agrochemicals, and other environmental contaminants induced, inhibited or delayed the recruitment of *Rhizobia* bacteria to host plant roots with the result that fewer root nodules are produced and lower rates of nitrogenase activity are seen [108]. However, the adsorption of these environmental pollutants to biochar has the effect of reducing toxicity to other soil microorganisms, thereby increasing microbial biomass, including free-living nitrogen-fixing bacteria such as *Bradyrhizobium japonicum* [40,109].

It has been postulated that biochar can influence net nitrification rates through the sorption of inhibitory substances, but the mechanisms are site-specific and complex. For instance, biochar can sorb, and therefore reduce, the activity of compounds that could inhibit nitrifying bacteria [110] or potentially reduce the complexation of nitrogenous molecules, such as proteins, into tannin complexes [102]. In addition, over time, surface functional groups on aged biochar alter its capacity for absorption of different enzymes, thereby affecting enzyme activity and substrates, for instance, absorbing NH_4^+ and reducing nitrification [107,111]. The effect of the sorption capacity of biochar has not been elucidated for all soils but could profoundly influence nitrification rates in organic systems. Hence, this remains another area that requires further investigation to ensure that biochar's different sorption mechanisms promote mineralisation processes such that crops are adequately supported.

3.5. The Influence of pH, Cation Exchange Capacity and Electron Shuttle Services

The pH of soil is critical because it affects plant nutrient availability by controlling the chemical forms of various nutrients and influencing the chemical reactions they undergo. For instance, phosphorus, molybdenum and calcium become increasingly unavailable with decreasing pH, with a corresponding decrease in crop productivity [112]. Biochar influences pH because it is generally alkaline due to its ash content and release of base cations, but also due to intrinsic alkaline organic functional groups [13]. However, the pH of a biochar is governed by both feedstock and pyrolysis conditions. Streubel et al. [14] found that the pH of herbaceous biochars was two units higher (9.4) than woody biochars (7.4) due to higher concentrations of ash in their study on contrasting biochar types (all pyrolysed at 350 °C). Yuan and Xu [15] found that pH was higher in biochars from leguminous feedstock with a pH range of 9.02 to 10.35 than in non-leguminous feedstock with a pH range of 8.00 to 9.24. Carbonates and organic anions of carboxyl and phenolic groups were the main alkalis but, again, this varied with feedstock.

As pH influences the chemical form and availability of substrates, it can affect change in both ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB) communities, thereby affecting mineralisation rates [113,114]. Accordingly, the addition of biochar has been shown to increase fixation rates, albeit to a lesser extent, and this capability appears to diminish over time [31,48].

pH, as amended by biochar, may have a greater influence on mineralisation rates, but the results are highly inconsistent and will depend on the existing pH levels of the amended soil. For instance, autotrophic nitrification generally occurs in neutral and alkaline soils because a critical enzyme, ammonia monooxygenase, uses NH_3 as a substrate rather than NH_4^+ with the balance affected by pH, with a higher pH favouring NH_3 [115]. Hence, although in an already alkaline soil, the addition of biochar resulted in a decrease in the number of nitrifiers [55]. However, an amended-acid soil resulted in a significant increase in the abundance of AOB correlating with an increased pH resulting from wheat biochar application [49]. Yet, the same study found no significant difference in the size of the AOA population with increasing pH, which correlates with the findings of [115]. This may be because AOA can be found in a wide range of soil pH, with some populations adapted to highly acidic soils. In another study, significantly lower net mineralisation rates have resulted from increased *Eucalyptus* biochar application due to decreased activity of the micro-organism community [116]. However, where there has been a decrease in nitrifiers, it has led to an increase in nitrification [55]. This increase may be because of biochar's effect on the air and water balance in soil (discussed below).

Again, as with mineralisation, the pH effect on denitrification is inconsistent [90,117]. For instance, Borken et al. [118] found a decrease in N_2O emission after liming of different forest soils, but Clough et al. (2004) found that WFPS had a greater bearing. However, Obia et al. [13] investigated the effect of two different types of biochar treated to remove alkalinity. They found that denitrification rates and gaseous products (NO, N_2O and N_2) were related to the increase in pH resulting from increased rates of biochar application. The untreated biochar suppressed NO and N_2O, but increased N_2 production, irrespective of the effect on denitrification rates. The treated biochar (which had been acid leached to reduce its liming effect) reduced or eliminated both its ability to suppress N_2O and NO production, apparently confirming the importance of altered soil pH as a result of biochar addition for denitrification.

For comparison, in a study which increased the pH of soil with ash applications (as opposed to biochar), there were no observable reductions in N_2O emissions [117]. The explanation proffered by Cayuela et al. [90] was the potential role of biochar as a reducing agent. This is because biochar may comprise manganese and iron—which readily function as electron acceptors [119]. Biochar may provide electron shuttle services, acting as an electrical conduit and facilitating electron transfer to micro-organisms. Hence, biochar would effectively compete with NO_3^- as an electron sink, thereby explaining a reduction in denitrification [90].

Biochar retains mineral ions in the rooting zone through its CEC. Feedstock type and pyrolysis conditions have an effect on the consequent negative surface charge of biochar with CEC including 3.8, 60.6, 137.6 and 254 cmol kg^{-1} for sugarcane bagasse, rice straw, chicken manure and peanut straw, respectively [120,121]. This surface charge results from carboxylate groups on the surface of biochar itself but also from exposed carboxylate groups of organic acids sorbed onto the biochar [122]. However, Wu et al. [123] found that, in the case of rice straw biochar, it was pyrolysis temperature, rather than residence time, which had a greater bearing on CEC. Higher pyrolysis temperatures generally cause greater condensation of aromatic structures resulting in aromatic carbon forms with less surface area and fewer oxidisable functional groups [124]. Therefore, not all biochars can raise soil CEC or oxidise to do so over time [120]. In fact, although many studies report an increase in CEC of soils amended with biochar [12], these are often degraded, poor soils with an inherently low CEC. For example, work done by Martinsen et al. [125] reported the influence of three different biochars on 31 different soils, which were all acidic and had a low- to medium-range CEC. Here, the addition of biochar was found to raise CEC as well as pH and exchangeable bases. However, where CEC increases over time, it may result in increased retention of NH_4^+ and, therefore, limit the supply of this substrate for other processes, causing a decrease in BNF [126,127].

Biochar CEC can have a direct effect on NH_4^+ leaching. Singh et al. [32] found that, over time, soils amended with biochar became effective in reducing NH_4^+ leaching but efficacy varied with pyrolysis temperature, with the high-temperature biochars decreasing

NH_4^+ leaching from both soils (Alfisol and Vertisol) by 55–93%, but low-temperature biochars decreased leaching by 87–94% in the Vertisol only. This may have been due to the reduced surface area in the biochar resulting from low-temperature pyrolysis [71,75].

Different biochars can have some level of anion exchange capacity (AEC). Maize stover, cellulose, alfalfa meal and albumin biochars ranged from 0.602 to 27.76 cmol kg^{-1}, respectively, and this increases with increasing pH [128]. These results may explain the reduction in NO_3^- leaching from biochar-amended soil treated with a biosolid where leaching of NO_3^- decreased to a level below control treatments [129].

3.6. The Influence of Inhibitory Substances

After pyrolysis of biomass, compounds toxic to micro-organisms may be present including polyaromatic hydrocarbons (PAHs) [130–132]. The presence of inhibitory substances due to biochar addition is of particular concern as once added to soil, it is almost impossible to remove, therefore any negative environmental consequences may be long-lasting. For instance, Anderson et al. [39] found that applying biochar to a silt-loam soil decreased the abundance of *Nitrosovibrio*—an AOB. As this is a rate-limiting step for nitrification, rates fell, which may be due to the introduction of inhibitory substances [33]. Wang et al. also found that phenolic compounds, which are retained by biochars, especially at low-pyrolysis temperatures, may inhibit microbial activity [133]. Furthermore, in their experiment with peanut shell biochar, one treatment with retained phenolic compounds and another without, they found that the presence of phenolic compounds likely reduced AOB abundance, thereby suppressing nitrification processes.

4. Summary of the Influence of Biochar on the Nitrogen Cycle

To conclude the above discussion, as the nitrogen cycle is governed by the activities of micro-organisms and biochar influences both micro-organism action and community composition, as well as other physical and chemical processes in the soil, it has a profound influence on fixation, assimilation, mineralisation and denitrification.

The main influences on fixation are through the promotion of mycorrhizal root colonisation due to the provision of carbohydrates that form part of the metabolisable fraction of carbon supplied by biochar or by providing physical protection from fungal grazers [48,68,69]. Biochar sorption capabilities may be hugely influential as they sorb nitrogen, reducing its availability in soil, thereby promoting nodulation [134]. It sorbs pollutants detrimental to micro-organism growth including fixers [40,109], but can also interfere with signal pathways, potentially interrupting nodule development [101,103]. Biochar can alter soil pH such that soil fixers benefit, although this process may diminish over time [135]. The provision of a metabolisable form of carbon, as well as recalcitrant forms, has been found to increase nitrogen-fixing organisms [31,48]. Figure 2 gives a summary of the substrates and products of fixation and how biochar influences the chemical and biological processes that lead to the products.

Biochar influences the assimilation of nitrogen into micro-organisms and plants via several mechanisms. Firstly, it can reduce leaching, keeping nitrogen in the rhizosphere and available for uptake. It can do this through the adsorption of NH_4^- or organic nitrogen onto biochar, intercalation or cation or anion exchange reactions [136]. Secondly, it improves soil water-holding capacity in some soils, which aids root and hyphal elongation and nutrient capture [68,135] even under conditions of high water evaporation stress [77]. Any change in pH brought about by biochar can either improve nutrient availability (and therefore leaching) or nutrients can become further unavailable [137]. In addition, the metabolisable carbon fraction can result in increased micro-organism growth and immobilisation of nitrogen [18,120,138,139]. These factors (captured in Figure 3) combine to create a complex soil ecosystem resulting in different rates of assimilation but also, therefore, different autotroph communities able to mineralise organic matter and continue the cycle.

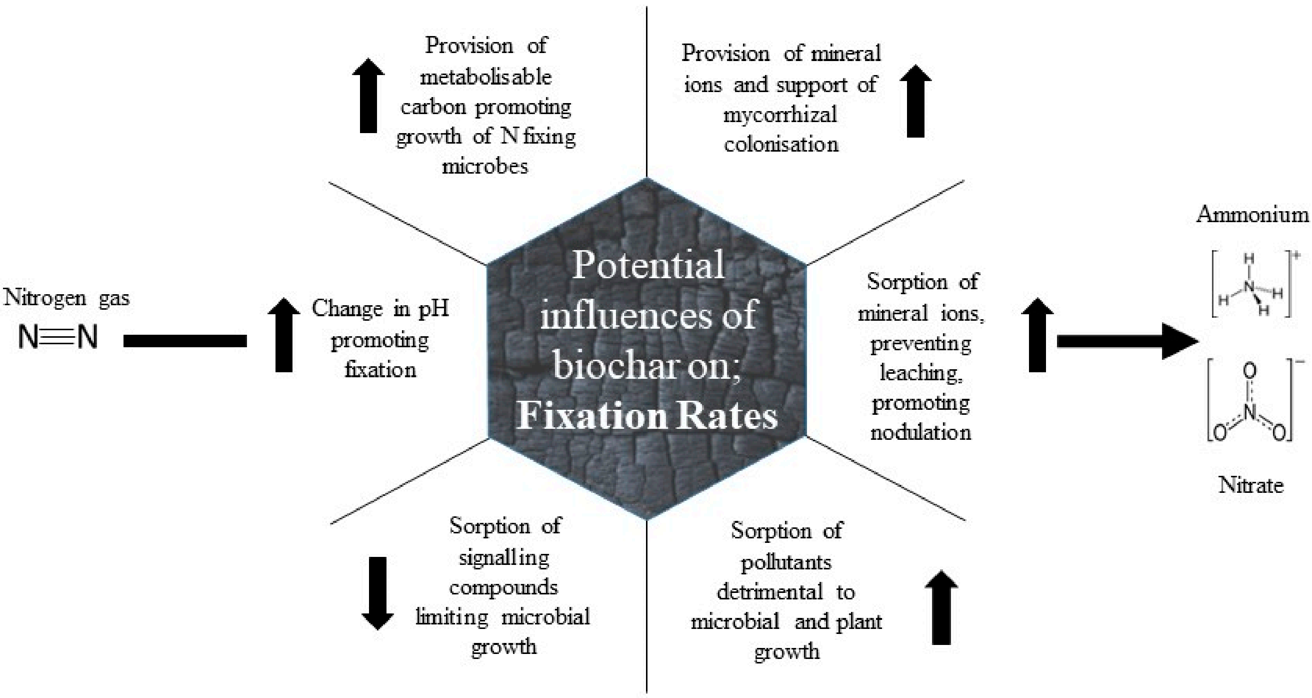

Figure 2. Potential mechanisms of biochar influence on fixation of atmospheric N_2 and production of inorganic nitrogen (arrows denote change in product formation rate).

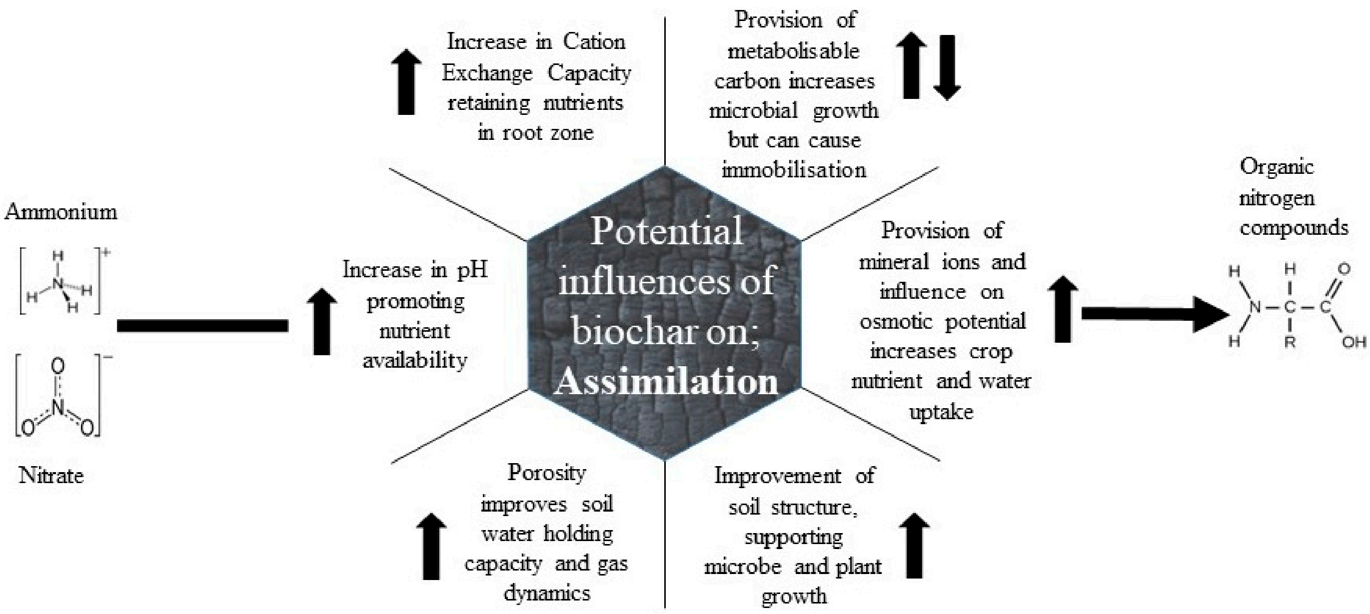

Figure 3. Potential mechanisms of the influence of biochar on assimilation of inorganic nitrogen and production of organic nitrogen, e.g., amino acids (arrows denote change in product formation rate).

Much research has been conducted on the influence of biochar on mineralisation rates and responses vary with soil type. However, the main mechanisms of influence include the provision of a metabolisable carbon, which influences micro-organism growth (Figure 4). For instance, as the process of ammonification is mediated by micro-organisms (usually *Bacillus* spp., *Proteus* spp. and *Pseudomonas* spp.) reliant on nutrient, energy and water resources, as well as communication mechanisms, and biochar can influence the rate and result of ammonification through its influence on soil structure and WHC, the provision and sorption of toxins, signalling compounds, nutrients and energy sources (that is, metabolisable carbon). However, studies analysing the effect of biochar on the transformation of NH_3 or NH_4^+ to NO_3^- or nitrification have revealed contrasting results. In some soils, there is no effect on nitrification rates [56,110]. However, in others, biochar promoted net nitrification [139,140].

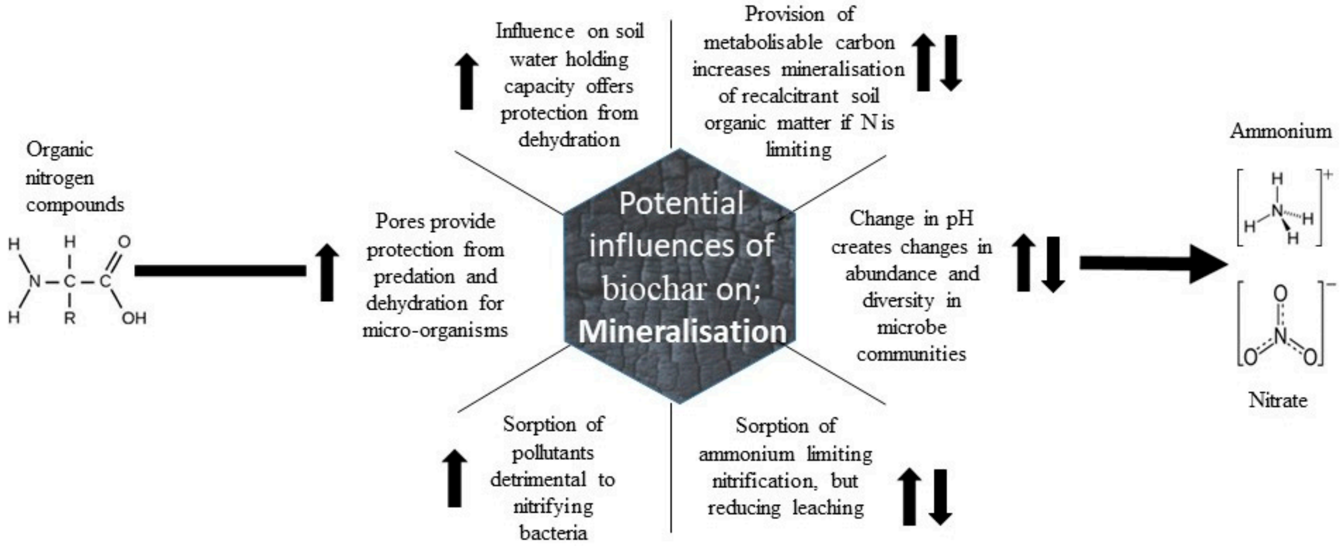

Figure 4. Potential mechanisms of the influence of biochar on mineralisation of organic nitrogen to non-organic forms (arrows denote change in product formation rate).

If nitrogen is limited in that soil, this could lead to the mineralisation of recalcitrant organic sources of nitrogen [28,39]. Any change in pH can affect the abundance and diversity of mineralising micro-organisms [39]. Sorption of substrates (NH_4^+) can limit mineralisation [136], but sorption of pollutants may benefit nitrifying populations [40]. Biochar pores may offer protection from predation and dehydration, thereby protecting mineralising populations [48,69].

The Influence of Biochar on Denitrification

The denitrification process is generally governed by several species of heterotrophic facultative anaerobic bacteria and archaea, which oxidise NH_3 and NH_4^+ via more than one enzymatic pathway [141]. The composition and genetic capability of the denitrifying micro-organism community and environmental conditions of a given soil dictate the pathways and partitioning of eventual nitrogen products. However, the facultative nature of the anaerobic bacteria, enzyme action and enzymatic pathway and the reduction in oxidised forms of nitrogen in response to an electron donor governs the mode of action biochar has on these processes. Hence, the mechanisms of the effect of biochar on denitrification known so far include the provision of metabolisable carbon and subsequent effect on soil aeration and the development of anaerobic microsites; the adsorption of substrates and products, for example, N_2O; pH; and the provision of electron shuttle services and toxic compounds (Figure 5).

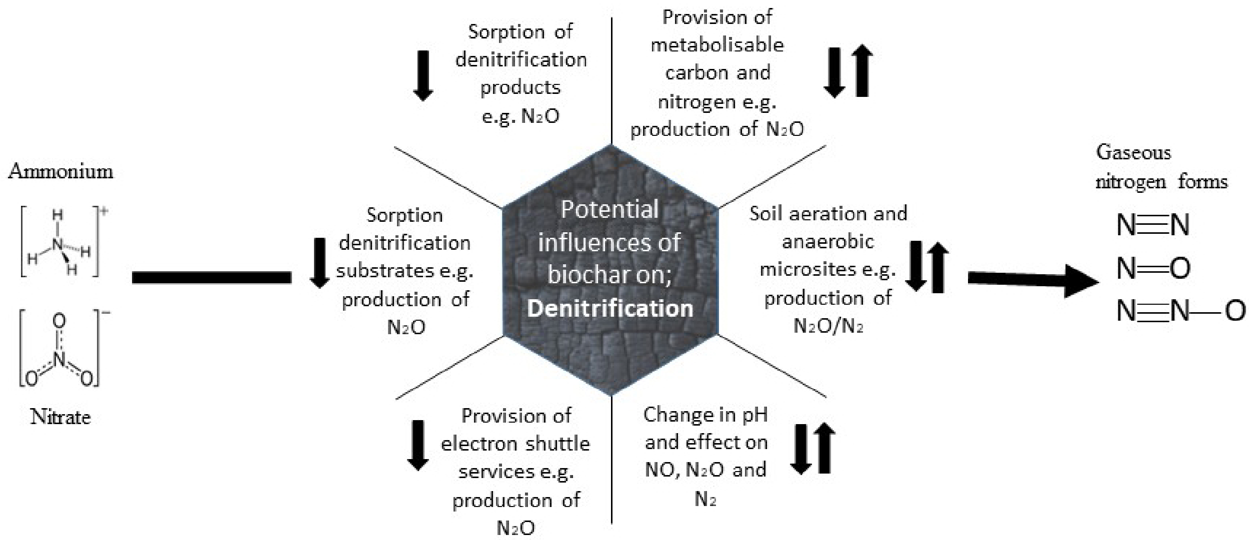

Figure 5. Potential mechanisms of the influence of biochar on denitrification of inorganic nitrogen (arrows denote change in product formation rate).

5. Conclusions

This review has assessed biochar along different parameters in terms of their influence on soil nitrogen transformations. Biochar's influence on fixation rates generally stems from its provision of two forms of carbon, metabolisable and recalcitrant, both of which have been found to impact the number of nitrogen-fixing organisms. Carbon forms also affect assimilation rates as a high metabolisable carbon fraction can result in nitrogen immobilisation, and incidences of a decrease in yield have been noted. This clearly has implications for the sustainable use of biochar in future, where agricultural yield is a consideration. However, biochar also changes pH, making nutrients more or less available for assimilation as well as affecting abundance and diversity of mineralising micro-organisms. Equally, biochar's ability to sorb pollutants may benefit nitrifying populations. Biochar also influences soil aeration and development of anaerobic microsites thereby influencing the release of denitrification products. However, the rates of such processes are dependent on biochar and soil type. Future studies should ensure that a detailed analysis of biochar feedstock, pyrolysis conditions and biochar characteristics are included, which would enable a greater understanding of the role of biochar on nitrogen transformations and should ensure sustainable deployment of this proven climate change mitigation tool. It is hoped that this review will serve as guidance for future studies and as a reference for students and practitioners alike.

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