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MINERAL NITROGEN AND BIOCHAR: ISOTHERM ADSORPTION AND SUBSTRATE-PLANT INTERACTIONS STUDIES

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CHAPTER 1

The role of biochar in increasing the use efficiency of reactive nitrogen in soil

1.1 The role of nitrogen in sustaining world food production

The world population was overall 7 billion people in 2012 and by the middle of this century it is expected to reach 9 billion (Faostat, 2013). While the world population increased by 129% in the last fifty years (Fig. 1.1), the global cereal production (excluding beer) increased by 151% from 1961 to 2009 (Fig. 1.2). The per capita consumption of food increase is the result of both the increased grain consumption and the increasing production in animal protein which diverts grain away from humans to domestic animals. The need for food and the limitations of the extension of the agricultural areas lead to the need to increase the yields of the cereal and, more in general, of crop production. Raising yield on existing farmland is necessary for "saving land for nature" (Tilman et al., 2002) and is one of the essential conditions to maintain the sustainability of agricultural practices. The term "sustainable", introduced by United Nations (UN, Brundtland report, 1987), is referred to the economic growth, it embraces the link between economy and ecology and it highlights the importance of the (sustainable) development as a prerequisite for peace, security and protection of the environment. The "sustainable agriculture" is defined as "practices that meet current and future societal needs for food and fibre, for ecosystem services, and for healthy lives, and that do so by maximizing the net benefit to society when all costs and benefits of the practices are considered" (Tilman et al., 2002). The usual business to raise yields is to increase the nutrient use (Nitrogen, Phosphorous and water amongst all) or to improve the nutrient use efficiency which is the ratio between yield and added nutrient according to Lopez-Bellido, L., Lopez-Bellido, R.J., Redondo, R., 2005.

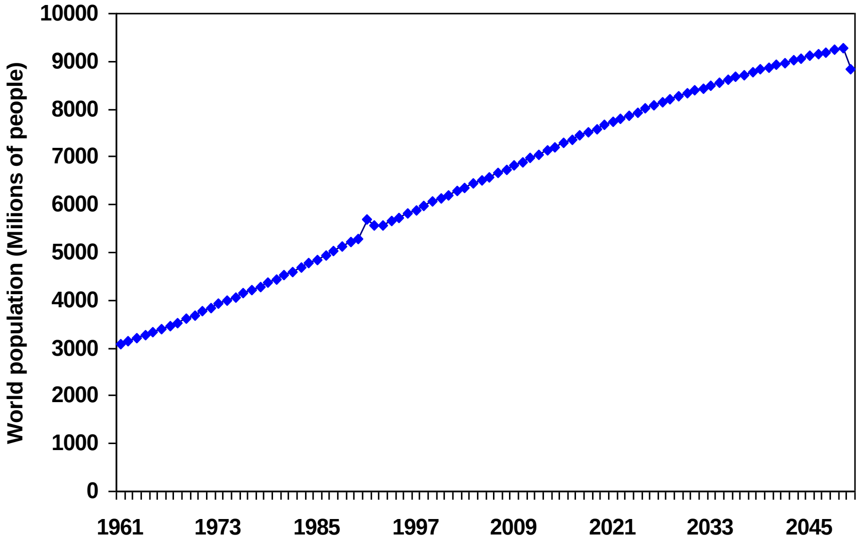

Figure 1. 1 World Total Population. Data elaborated from FAO statistical databases (Faostat 2013)

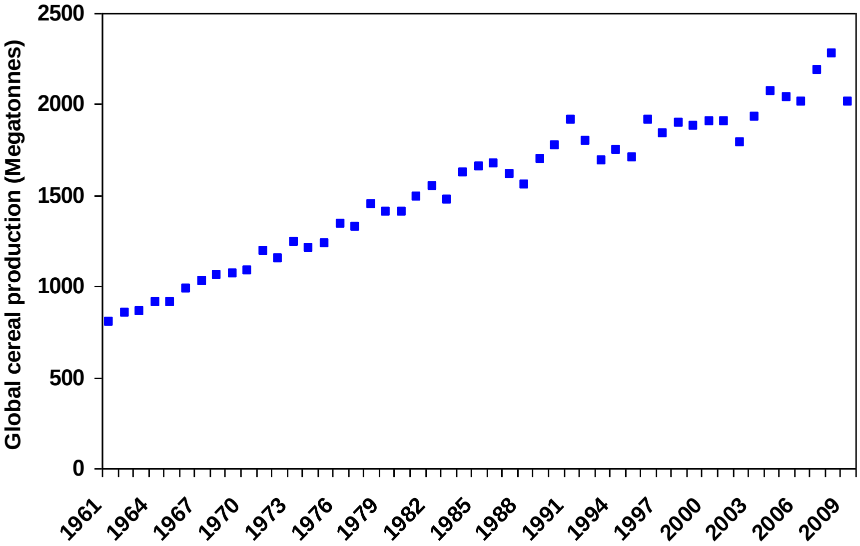

Figure 1. 2 Global cereal production in the last 50 years. Data elaborated from FAO statistical databases (Faostat 2013**)**

1.2 Biological and anthropogenic inputs of reactive[1] N in soil ecosystem

Nitrogen (N) is the very basic building block for cell metabolism, such as amino acids and nucleotides. Its content ranges from 2 to 10% of living organisms by weight. It accounts for roughly 78% of Earth's atmosphere on a molar basis as dinitrogen (N_2). However, despite the atmospheric abundance of molecular nitrogen, this form is kinetically inert (unreactive), hence not available either to plants or animals.

In soil, nitrogen occurs in both organic and inorganic forms. The organic forms represent 90-99% of the soil N and could be found in (i) litter partially decomposed residues, consisting of undecayed plant and animal residues, and (ii) in soil organic matter or humus (Kelley and Stevenson, 1995). Organic forms of soil N are not directly available for plants. To be usable by plants, organic N must be converted into inorganic N, by biologically mediated decomposition of soil organic matter processes. Ammonium (NH_4^+) and Nitrate (NO_3^-) forms constitute the most important fractions of inorganic (mineral) N produced by such processes (e.g. Taiz and Zeiger, 1998).

Biological Nitrogen Fixation (BNF), is another important process which substantially contributes to the reactive N inputs of terrestrial and marine ecosystems. Terrestrial BNF is carried out by free living or symbiotic N_2-fixing micro-organisms (most of them Bacteria) able to capture atmospheric N_2 and to deliver it in the form of NH_4^+. BNF contributes with 107 and 31 Tg year^{-1} of reactive N produced in terrestrial and cultivated ecosystem respectively (Galloway et al., 2004). N could be emitted back to the atmosphere as NO_x, NH_3, N_2O and N_2, the latest as the end product of denitrification .

Human activity has profoundly altered the global biogeochemical cycle of N, having approximately doubled the rate of N input into the terrestrial N cycle, which resulted in the fixation of an additional 150 Tg N year^{-1} (e.g. Vitousek *et al.*, 1997; Galloway et al 2004). The anthropogenic inputs derived i) by the industrial fixation

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[1] The term reactive nitrogen as used in this thesis includes all biologically active, photochemically reactive, and radiatively active N compounds in the atmosphere and biosphere of Earth. Thus reactive nitrogen includes inorganic/mineral reduced forms of N (e.g. NH_3 and NH_4^+), inorganic/mineral oxidized forms (e.g. NO_x, HNO_3, N_2O and NO_3) and organic compounds (e.g. urea, amines, proteins) by contrast to unreactive N_2 gas (i.e. Galloway et al., 2008).

of N, with the aim to produce N fertilizer, mostly via the Haber-Bosch Process of NH_3 production; ii) by the promotion of BNF with the cultivation of legumes and the production of rice; iii) by the emission to the atmosphere of NO_x from the combustion of fossil fuel (Tilman et al., 2002; Galloway, 1998; Galloway 2004). The human induced BNF was the most important mechanism of introducing new N into agricultural ecosystem up until 1960s or 1970s and, on average, its contribution is estimated on 40 Tg N year^{-1} (Galloway, 1998). By 1970 the dominant process of anthropogenic N fixation was the commercial production of N fertilizer, realized initially through the Haber–Bosch (1909) synthesis of ammonia (NH_3). To date the energy used in the world for the production of fertilizers is 1.1% of the total annual use of energy and is equivalent to 5850 PJ. The energy required for production of N fertilizers accounts for over 90% of the total energy input of fertilizers' life cycle (Dawson and Hilton, 2011).

1.3 N use efficiency is dependent on soil immobilization and losses of mineral N

The global use of N fertilizer between 1960 and 2002 increased seven fold (Faostat, 2013; Fig. 1.3) and further increases in N fertilizer use are expected by 2050 due to increasing food/feed demand.

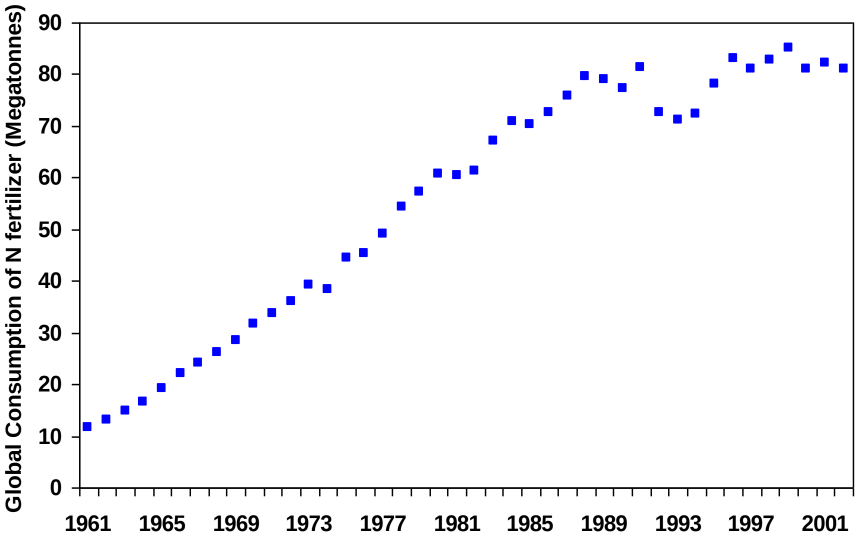

Figure 1. 3 Global consumption of N fertilizer. Data elaborated from FAO statistical databases (Faostat 2013**)**

Beside the fact that anthropogenic N inputs support the agricultural productivity, it must be considered that only 30-50% of applied nitrogen fertilizer is taken up by crops (Smil, 1999). One factor affecting the efficiency by which fertilizer N is taken up by crops is the conversion of mineral N into soil organic matter by the progressive stabilization of it into more resistant humus forms. ^{15}N studies demonstrated that from 20 to 40% of the fertilizer N added to agricultural crops of temperate-zone soils is typically incorporated into organic forms during the first growing season (Kelley & Stevenson, 1995). Moreover, no more than 15% of this residual N becomes available to plants during the second growing season, and availability decreases even further for succeeding crops (Kelley & Stevenson, 1995). Some percentage of the newly immobilized N is susceptible to be shortly mineralized, but a significant portion is slowly decomposed like the native humus N.

The fraction of N fertilizer distributed to the soil which exceed the plant demand and is not immobilized in organic compounds leads to nutrient losses and inadvertent addition to other ecosystems. Many subsequent treats arise: the leaching rates of nitrate from surface to groundwater can increase considerably, atmospheric emissions of N_2O, NH_3, NO_x will lead to N deposition and acidification of soil (Powlson, 1994), which may finally cause shifts in the species composition of both terrestrial and aquatic ecosystems (Tilman, 1988; Smith, 1999).

The problems of NO_3^- leaching and contamination of surface and groundwater are particularly severe in developed countries, where the use of N fertilizers and organic waste is widespread. Leaching and runoff of N to ground and surface waters may occur from uncovered and unsealed manure storage systems and from agricultural fields, overall in permeable and highly weathered soil. Surface runoff occurs when the rainfall intensity (mm/h) exceeds the infiltration rate (mm/h) into the soil. High concentrations of NO_3^- in drinking water is deemed harmful to human health, particularly for infants less than 1 year old (Di et Cameron, 2002). It can interfere with the transport of oxygen in the blood, causing methemoglobinemia (blue-baby syndrome). To protect human health, the World Health Organization in 1984 has established drinking water standards, limiting NO_3^- concentration to a maximum of $10 \div 11.3$ mg NO_3^-–N L^{-1}. Moreover, high concentrations in surface water bodies, rivers, lakes and estuaries can cause the deterioration of surface water quality, finally resulting in eutrophication phenomena (e.g., algal bloom, fish poisoning, oxygen depletion) concerning aquatic ecosystems. An aquatic system is classified as eutrophic when the total N concentration reaches 0.4 ÷ 0.6 mg NO_3^-–N L^{-1}.

A series of environmental policies have been implemented in the European Union (EU) to decrease N-losses from agriculture: the Nitrates Directive (ND), adopted in 1991 (Council Directive 91/676/EEC), is one of the main policies. It aims to reduce nitrate leaching from agriculture and compel all EU member states to designate the so-called Nitrate Vulnerable Zones (NVZs). NVZs are regions where the nitrate concentrations in ground and drinking water amount to 50 mg L^{-1} or more. According to article n°10 of ND, member states are required to adopt mitigation strategies for water pollution promoting implementation of a Code of Good Agricultural Practice and an Action Programme. Finally they have to monitor surface and ground water quality, and to submit assessment reports to the European Commission every 4 years (Monteny, 2001). Consequently to the implementation of ND principles, the mineral fertiliser consumption had a progressive reduction in the early 1990s (Fig. 1.3) and stabilised during the last years in the EU-15, but across all 27 Member States nitrogen consumption has increased by 6% during the same period (Faostat, 2013).

High levels of mineral nitrogen in soils contribute to the emission of nitrous oxides (N_2O), ammonia (NH_3), nitrogen oxides (NO_x) from croplands (Fig.1.4). Nitrous oxide is an important greenhouse gas, despite the low atmospheric concentration (~ 320 ppb) it has a long lifetime in the atmosphere and a high global warming potential (GWP) estimated as 298 times higher than the one of CO_2 over a 100 years period (Forster et al., 2007). Emissions of N_2O from agricultural fields are mostly associated with denitrification following fertilizer application when soil moisture reaches field capacity, and to a lesser extent with nitrification (Bouwman, 1990). Factors controlling denitrification include the presence of an energy source for the denitrifying bacteria (mostly metabolizable organic carbon), anoxic soil conditions, and the presence of nitrate. The main factors controlling the nitrate soil production (e.g. nitrification) are: the content of dissolved ammonium (NH_4^+), the pH> 7, oxic soil conditions, and high temperature. The United States are responsible for the highest amount of N_2O emissions, followed by Russian Federation, France and Germany. According to the last available data (2006) Italy is at the 7th place in the ranking of Countries with higher emissions of N_2O (United Nations Statistic

Division, UNSD, 2010). N_2O soil emissions are responsible of more than 50% of total greenhouses gases produced in cropped areas.

Emissions of ammonia (NH_3) are mainly (74%) derived from feces and urine in housing and manure storage systems and from excreta of grazing animals voided on pastures. A further 13% of NH_3 emissions derived from fertilizers production and application, in particular from the application of urea fertilizer and to a less extent of calcium ammonium nitrate (ECETOC, 1994).

Nitrogen oxides (NO_x), i.e. mono-nitrogen oxides (NO) and nitrogen dioxide (NO_2), are produced from the reaction of N and O_2 gases in the air during combustion, especially at high temperatures and from (Zheng H.)nitrification of reactive N in the soil. An emitted molecule of nitrogen oxide can first cause photochemical smog and then, after being oxidized in the atmosphere to nitric acid and deposited into the ground as acidic rainfall, can lead to ecosystem acidification and eutrophication (Gruber & Galloway, 2008). According to UNSD report 2010, the maximum amount of NO_x emissions are produced in the United States (13.94 MegaTonnes), followed by Paraguay (6.91 MegaTonnes) and the Russian Federation (5.07 MegaTonnes). Italy is at the 17th place in the ranking of countries with higher emissions of NO_x, however the emissions reported in 2007 were strongly reduced (-47%) compared to 1990.

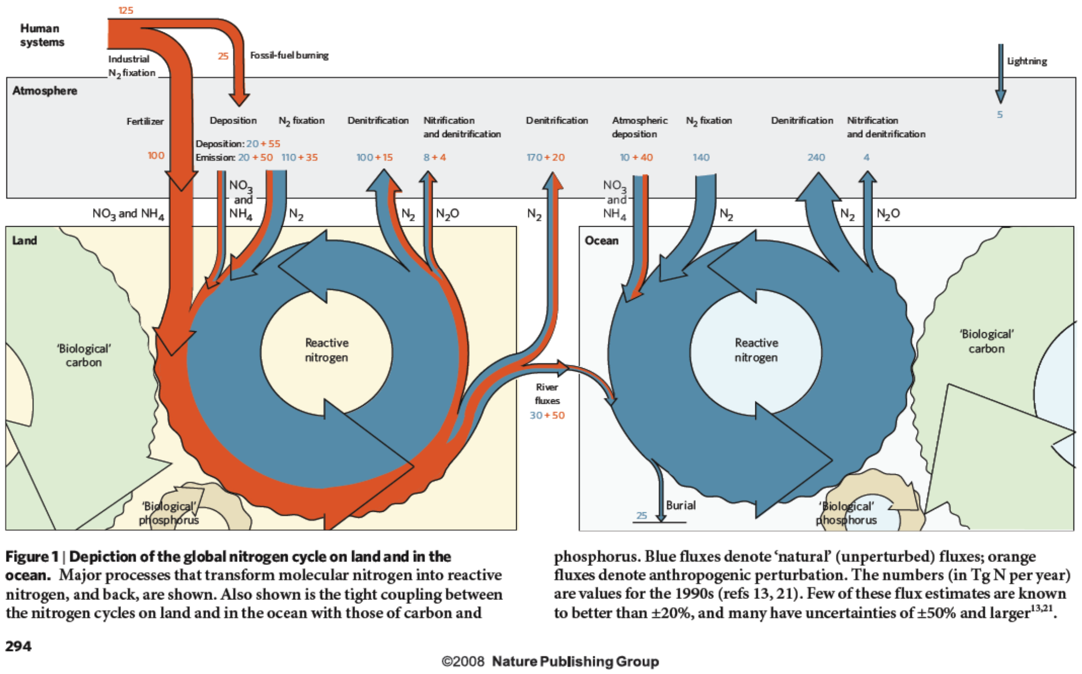

Figure 1 | Depiction of the global nitrogen cycle on land and in the ocean. Major processes that transform molecular nitrogen into reactive nitrogen, and back, are shown. Also shown is the tight coupling between the nitrogen cycles on land and in the ocean with those of carbon and phosphorus. Blue fluxes denote 'natural' (unperturbed) fluxes; orange fluxes denote anthropogenic perturbation. The numbers (in Tg N per year) are values for the 1990s (refs 13, 21). Few of these flux estimates are known to better than ±20%, and many have uncertainties of ±50% and larger[13,21].

Figure 1. 4 Natural and anthropogenic components of the N cycle. Gruber and Galloway, Nature 2008

Losses of N, towards downstream ecosystem, atmosphere and waste, has biogeochemical and financial impacts.

Optimising the N-use efficiency, by minimising N-losses to air and water, by retaining reactive forms of N for a longer period into the soil or within biogeochemical cycles, by improving biological N fixation and subsequent use or by recycling farm-generated manure, is an absolute requirement to sustain growing population food demand.

To achieve this goal a potential new opportunity is into the use of biochar.

1.4 The biochar strategy to optimize the N use efficiency

1.4.1 Biochar general definition and scopes

Biochar (BC) is a solid by-product, derived from the thermo-chemical conversion of renewable biomass (feedstock) in a low or zero oxygen concentration environment at temperature between 300 and 1000 °C (Verheijen et al., 2010). Pyrolysis and gasification are the technologies used to produce biochar and/or co-produce biochar and energy. Energy (thermal or electrical) derives from the combustion of the other by-product called ‘syngas’ (a mixture of vapour, H_2, CH_4 and CO) produced during the thermo-chemical conversion.

Feedstocks used to produce BC could derive from different wasted biomasses (i.e. woody, construction and demolition waste, municipal solid waste, refuse-derived fuels, slurries, bedding matter, manures, sewage and paper sludge). In this case it is possible to speak of biochar from ‘non virgin biomass resources’. On the other hand it is intended for ‘virgin biomass’ (e.g. wood, chips, straw, coconut shells, peanut shells and rice husks) where that does not involve chemical or biological transformation, amendment or treatment (Shackley and Sohi, 2010). In each of these cases, excepted for woods, biochar is made using materials otherwise unusable and of no value in themselves, hence wastes. The organic waste disposal, together with the production of energy (flaring syngas) are the two main objectives of the pyrolysis process. Further, the production of biochar is largely recognize as a strategy to sequester atmospheric carbon dioxide (CO_2).

Biochar is composed by a stable, recalcitrant organic carbon structure, which is highly resistant to decomposition even when is applied in to the soil. For this reason it is suggested as soil amendment as it is able to ameliorate some characteristics of cultivated soil, promoting often their crop yield (Lehmann and Joseph, 2009). The purpose of the production, that is the intent to incorporate this C-rich compound in the soil, distinguishes biochar from other kinds of “black carbon” (Lehmann and Joseph, 2009). Different forms of black carbon can be differentiated by the oxygen to carbon ratio (O:C) in the residual product (Spokas, Novak, Venterea, 2012; Fig.1.5).

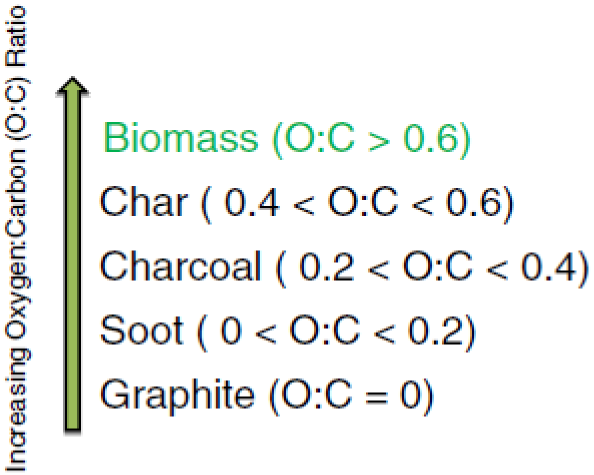

Figure 1. 5 Illustration of the various material forms within the black carbon continuum as defined by the range in the oxygen to carbon (O:C) ratio. From: Spokas, Novak, Venterea (2012).

The “biochar system” is defined as a “win win win” strategy as, in the future, it is aimed to use it in order to (i) substitute fossil fuels used for energy production; (ii) sequester C into a semi-permanent organic matter; (iii) increase soil fertility mitigating the use of non-renewable resources used for food/feed production. The potential of the “biochar strategy” has been largely described in the last decade’s scientific literature (Lehmann and Joseph, 2009; Verheijen et al., 2010), and its effect has been studied in several type of soil and weather conditions. Unfortunately, the research on this topic is almost in a preliminary stage of observation and description of the effects, derived from the biochar application into the soil. The effects on “biotic” and “abiotic” processes involved by BC addition to the soil, has not yet been well clarified (Jeffery et al., 2011).

1.4.2 The need for a qualitative definition of biochar

Biochar properties can be highly heterogeneous, since they depend on the feedstock and/or on the pyrolysis conditions used to produce biochar (Fig. 1.6). Such heterogeneity and complexity of biochar properties related to different production tecnologies, and the diversity of functions claimed for BC’s services, is hampering to identify a “one size fits all” biochar. However, this great variability is also a substantial source of wealth, because it may provide biochars with properties that are best suited to a particular scope, to solve specific site quality problems (Novak and Busscher, 2011).

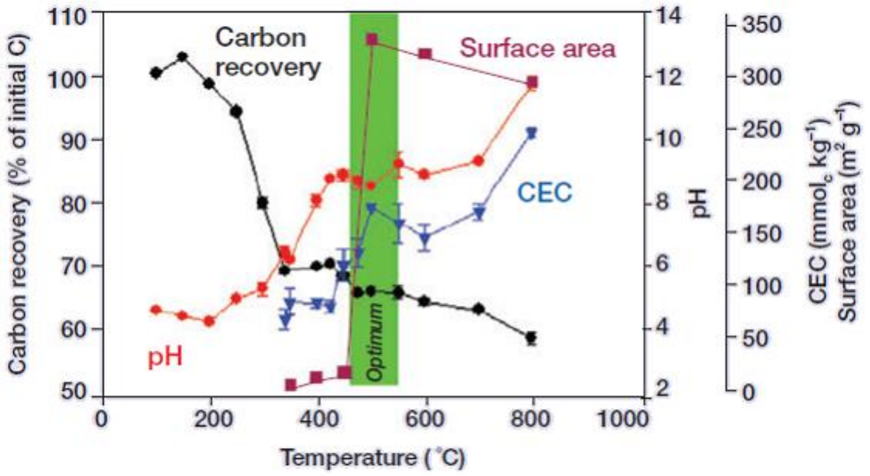

Figure 1. 6 Biochar physical-chemical characteristics change with pyrolysis temperature. From Lehmann 2007.

At the moment several biochar researchers, from academia and private commercial companies, are trying to define universally valid analytical protocols, which use will allow to certificate the best, sustainable and effective chemical-physical characteristics of biochar. The first finalized attempt in this direction was made by the International Biochar Initiative (IBI), which began work on the “IBI Biochar Standards” in May 2009. Afterwards, also in Europe two documents, intended to be used by producers, end-users and environmental regulators of biochar, have been published. The first version of the “Guidelines of the European Biochar Certificate” edited by the “European Biochar Foundation” headed by Schmidt H. P. was published the 1st January 2012. Almost contemporarily the “Biochar Quality Mandate” was published in the UK (last version updated on 15th June 2013) which provides the criteria by which a good quality biochar product, safe to use, can be evaluated with reference to the UK context. To date, nevertheless, only three European Countries, Switzerland (since 23 April 2013), Hungary and Austria, allow the use of certified biochar in agriculture as soil amendment.

1.4.3 Biochar and the soil N-cycle

The mechanisms underlying the increased plant productivity following the addition of biochar to soils have not yet been completely clarified. Among the most accredited hypothesis one concerns the impact of biochar on the nitrogen cycle (e. g. Clough et al., 2010; Spokas, Novak, Venterea, 2012) and, particularly, the biochar capability to reduce N environmental losses, thus enhancing the plant availability and, therefore, the use efficiency of this nutrient. The N cycle is a dynamic (time dependent) system, with numerous processes (rate variables) regulating the fluxes and the transformation of N between the different chemical forms (state variables or pools) in which this macro-nutrient is present in the soil (Fig.1.7).

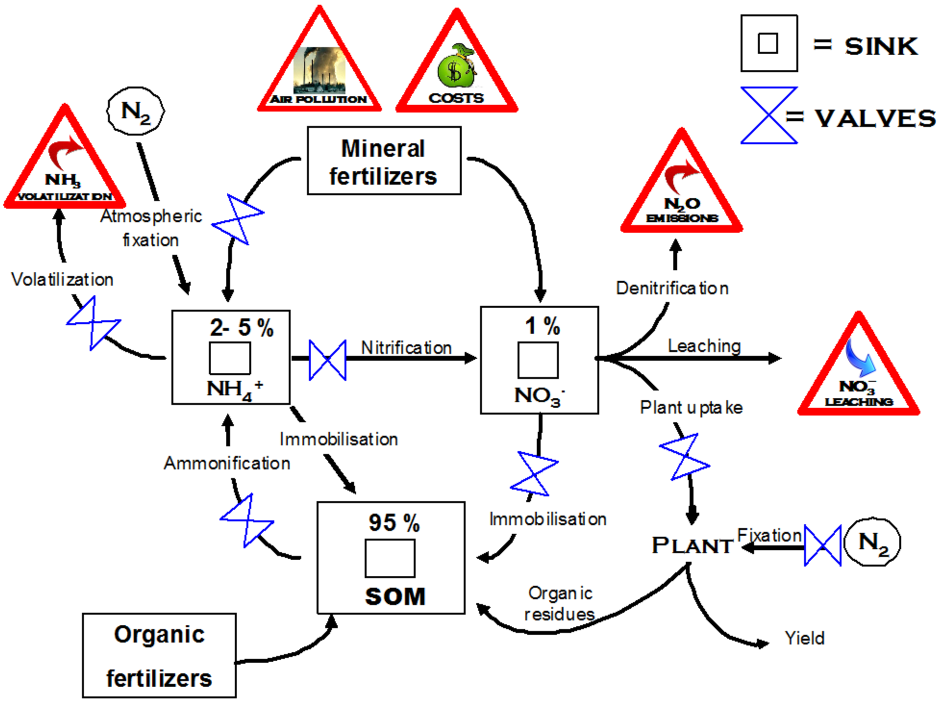

Figure 1. 7. The N cycle, its environmental impacts and the hypothesised fluxes in which biochar is supposed to interfere with it.

Biochar could interfere, directly or indirectly, with these processes affecting amount and quality of N in the soil. For instance, it was demonstrated that moderate rates of BC additions significantly enhance the biotic nitrogen fixation (BNF) of common *Phaseolus vulgaris* L. (Rondon M. A. et al., 2007). Until now, several studies have been conducted trying to define the ways in which interactions between biochar and soil occur, nevertheless results are not agreeing among conclusions and the effects are not constant, due to the various nature of BC and of soils in which biochar was added. As an example, contrasting hypothesis coming from different studies are presented here:

1) BC induced plant N deficiencies because its high C/N ratios could enhance N immobilisation (organication) in soil (Atkinson *et al.*, 2010).

2) the recalcitrant nature of BC and the limited availability of labile C-compounds, could greatly limit the N immobilisation (Chan and Xu, 2009).

It must be taken into account that, even if BC alters only one mechanism of the soil N cycle, this change would trigger a "cascade effect" with immediate impacts on microbial community presence and composition, plant N availability and yield (Clough et al. 2013). For instance, the mineralization of soil organic matter (SOM) is strictly connected to the nitrification process, since it provides the substrate for nitrifying microorganisms which oxidize $NH_{3,}$ firstly to nitrite (NO_2) and finally to nitrate (NO_3^-). Hence, if biochar increases the mineralization rate of SOM, it will cause, indirectly, an increase in the pool of mineral N into the soil, which could results i) in more available N for plants ii) in an higher presence of NO_3^- and dissolved organic nitrogen (DON) susceptible to water leaching. On the other hand, the subtraction of soluble N forms caused by shifting the equilibrium towards the synthesis of new organic matter (N immobilisation), might results in a reduced available pool of mineral N with the consequence of probable N deficiencies for plant's nutrition. Thus, if the hypothesis of Atkinson *et al.* (2010) will be confirmed, it will possible to affirm that biochar is responsible for the reduction of the mineral N pool. According to the "equation of nitrate problem" one could state: less N availability equal less vulnerability and major N deficiencies for plant (Addiscott, 2005; Oenema et al., 2009). Nevertheless, plant N deficiencies following biochar incorporation into the soil were rarely observed, on the contrary most authors observed an increased availability of NO_3-N in the soil, after the addition of biochar (e.g. Van Zwieten et al., 2010).

1.4.4 Influences of biochar on organic N of forest and agricultural soils

It has been demonstrated that the addition of biochar could influence both mineralization and nitrification processes in the soil. Several studies carried out in forest soil observed a significant increase of the net mineralization and nitrification rate, which doubled the nitrate concentration in presence of charcoal compared with the untreated soil (e.g. DeLuca et al., 2006). At now, three hypothesis have been suggested to explain the "priming effect", i.e. the capability of BC to stimulate the mineralization of SOM, in forest soil. According to the first one, biochar or charcoal adsorbs N-poor organic compounds, such as phenols, which stimulated N immobilization. So BC indirectly promoted N mineralization (i.e. increase of net ammonification and nitrification) in forest soil (DeLuca et al., 2006). The second hypothesis suggests that BC (charcoal) adsorbs organic molecules which act as inhibitors of nitrification (such as monoterpenes), therefore shifting the equilibrium from immobilisation toward nitrification process. The third hypothesis suggests the BC induced increase in pH as responsible for the enhanced nitrification in acid or sub-acid forest soils (Ball et al., 2010). In fact an increased pH potentially provide a more favourable habitat for nitrifying organisms, in particular Ammonia Oxidising Bacteria (AOB). Furthermore, the first key enzyme of nitrification pathway, ammonia mono-oxygenase, uses NH_3 as substrate rather than NH_4^+ and the soil content of NH_3 is positively related to pH (Suzuki et al., 1974).

However the consequences of biochar addition in forest soil are not congruent with the effects obtained in agricultural soils. The addition of biochar in agricultural soil or grassland, which are not characterized by a high level of phenols and/or monoterpenes, and that already demonstrated high levels of nitrification, resulted in an insignificant or either negative priming effects, overall when the amendment is added at low rates (Dempster et al., 2012). Nevertheless, there was some evidence according to which biochar could accelerate, also in arable soil, the gross mineralization rate both by stimulating the mineralization of the recalcitrant N-pool (N_{rec}) to NH_4^+ and by favouring a faster immobilisation rate into labile N-pool (N_{lab}) fraction of the inorganic N (NH_4^+ and NO_3^-) present in the solution of the soil, due to the high C/N ratio of the labile-C compounds added with biochar (Nelissen et al., 2012). The priming effect of biochar was proportional to its application rate into the soil (Dempster et al., 2012) and negatively related to the temperature of biochar production (Nelissen et al., 2012). These two factors are, in fact, responsible for the labile C content in BC, which is the preferred substrate by soil microorganisms. The study carried out by Nelissen et al. (2012) remains until now, the only one in which, in an intensively managed arable soil, beyond the increasing of the gross mineralization rate, it was observed also an enhance in the gross nitrification rates (GNR). The authors suggested that the increase in the pH due to BC addition in an acidic soil (pH = 4.8) was responsible for the increase in the GNR. Thus, the GNR was positively related to the temperature of BC production, and BC produced at 550°C highly stimulated the GNR compared to biochar 350°, which pH was 1.5 point lower than the first mentioned, confirming the hypothesis suggested by Ball et al. (2010) to explain the enhanced nitrification in forest soil.

1.4.5 Influence of biochar on mineral N in the soil

In 2009 Spokas and Reicosky compared the behaviour of several biochar amendments across multiple arable soil type and hypothesized that a decreased soil microbial activity (thus also a reduced nitrification activity) was at the basis of the reduction of N_2O emissions observed in all the combinations of treatments. A subsequent study suggested the ethylene released by BC as responsible of the negative effect on soil microflora activity, including nitrification (Spokas, Baker, Reicosky, 2010). Other studies are not in agreement with these results and did not observe any effect of char addition on soil microbial biomass, neither on net nitrification activity (Zavalloni et al., 2011; Ventura et al., 2013).

Castaldi et al. (2011) and Clough et al. (2010) found that, despite a first period in which the emission of N_2O were even stimulated by the presence of biochar, the cumulative fluxes of N_2O at the end of the experimental period did not differ between biochar amended soil and control soil. Similar results were obtained by Yanai et al. (2007) who examined the effects of charcoal on N_2O emissions caused by rewetting of air-dried soil in the laboratory. They hypothesised that charcoal, by absorbing water, might improve the aeration of the soil, leading to a suppression of N_2O production, stepwise reduction of NO_3^-, NO_2^- and NO. Nevertheless they found that this behaviour of charcoal is strongly dependant on the initial moisture and aeration conditions of the soil. Castaldi et al. (2011), although not observing a significant suppression in N_2O emission, reported a significant decrease in the amount of extractable NH_4^+-N in samples treated with biochar compared to the control plots. Indeed, the reduction of the mineral N-pool, susceptible to nitrification or denitrification, could also result from the chemical adsorption of NH_4^+ or NH_3 by BC (e.g. DeLuca et al., 2009).

NH_3 reduced volatilization were observed either following the surface application of nitrogenous fertilisers and ruminant urine deposition (Steiner et al., 2010) either during the composting of manure (Tagizadeh-Toosi et al., 2012 b) if biochar was previously added to the system. Among the mechanisms responsible of

NH_3 reduction the formation of ammonium bicarbonate (NH_4HCO_3) was suggested which occurs in presence of NH_3 and CO_2 (Li et al., 2003). This process, when occurs in presence of a biochar material produced at 400°C, results in the formation of a white powder on biochar surface (Day et al., 2005). Another opportunity to explain the reduction of NH_3 volatilization is that biochar may sequester it in an NH_4^+ form (Tagizadeh-Toosi, 2012 a). This last process is reversible thus biochar adsorbed ammonia is available for plants (Tagizadeh-Toosi, 2012 a). This fact led the hypothesis that biochar can act as a transient store of plant available N, thus extending the time of N availability and increasing the amount of N retention in the soil (Steiner et al., 2008).

Beyond the reduction of N_2O emissions and NH_3 volatilization, many authors observed a strong reduction in leached mineral N as affected by biochar incorporation into the soil. Nevertheless the biochar effect on N leaching is also highly dependent on biochar characteristics, e.g. feedstock type and the pyrolysis conditions (Yao et al., 2012). Results referred to this last interaction are not consistent. Some of them report no or even negative effect of biochar in reducing the N-leaching (e.g. Singh et al., 2010; Major et al., 2012) or observed only a reduction of the ammonium leaching while no effect was detected on nitrate leaching (Ding et al., 2010). A significant loss reduction of NO_3-N (Ventura et al., 2013) or of both NO_3-N and NH_4^+-N forms was also observed (Yao et al., 2012). Biochar effects on NO_3 leaching was often measured using small pots or soil columns (e.g. Laird et al., 2010; Zheng et al., 2013) or, seldom, shallow lysimeters (Bell and Worral, 2011) or soil bags distributed in the top layer of the soil (Ventura et al., 2013). Studies in larger and deeper lysimeters, with both plants and standard fertilizer practices included, are required (Clough et al., 2013).

1.4.6 The adsorption of mineral N onto biochar

The reduction of mineral N concentration in leachate is attributed more to a modification of the microbial cycle rather than an higher adsorption (Knowles et al., 2011; Beck et al., 2011). Nevertheless the capacity of adsorption of various forms of N on biochar was hypothesised by several authors. Attempts of batch sorption laboratory experiments performed at the scope to clarify the sorption of different N reactive forms onto biochar were recently carried out and published. Most of these studies, nevertheless, were performed at room temperature and left unexplained numerous aspects of the sorption mechanisms of N onto biochar (Hollister et al., 2013; Saleh et al., 2012). The surface charge of biochar is one of the most accredited reason for the sorption of nutrient onto biochar and, for its characteristics, is supposed to be more effective for cationic (e.g. Tagizadeh-Toosi, 2012a; Yiobe et al., 2004) rather than for anionic species (Hollister et al., 2013). Direct adsorption of NO_3-N on charcoal surface were observed by Mizuta et al. (2004) and by Yao et al. (2012). In fact, even if biochar particle surfaces are characterized mainly by negative charge (Lehmann et al., 2011), anions may be attracted by bridge-bonding with divalent cations such as Ca^{2+}, Mg^{2+} or other metals like Al^{3+} or Fe^{3+} (Mukherjee et al., 2011). The use of charcoal / biochar as adsorbent of ammonia, ammonium and amine gases was often suggested as substitute of activated carbon, as BC is significantly cheaper than activated carbon because it does not require a costly second activation step (Steiner et al., 2010).

BC surface properties depend on the temperature at which the BC is produced (Fig.1.6) and on the aging of BC surface. CEC increases with pyrolysis temperature but while it was demonstrated that an increased NO_3-N adsorption on biochar was due to increasing temperature of biochar formation (Yao et al., 2012; Mizuta et al., 2004) no apparent pyrolysis temperature trend was observed in ammonium sorption (Yao et al., 2012). It has been demonstrated that BC slowly oxidises over time and its surface functional groups change as the particle gains greater negative charge, increasing its hydrophilicity (Ventura et al., 2013; Cheng et al., 2006, 2008).

The negative charges developed on biochar surface can buffer acidity in the soil, as organic matter does in general. Also changes in environmental pH conditions have been suggested as involved in the reduction of the pool of $N\text{-}NH_4^+$ in soils after biochar addition (Ding et al., 2010). Increasing soil pH, in fact, determined an increase of soil CEC particularly in the organic matter fraction of soils (Silber et al., 2010), and have a direct effect on soil nutrient retention.

1.5 Objectives and thesis outlines

The general purpose of this thesis was to focus on biochar and N chemical interactions, particularly referring to ammonium-nitrogen (NH_4^+-N) adsorption onto biochar, in order to clarify whether this mechanism could be responsible for the biochar effects on the N cycle in the soil eventually altering the N use efficiency of plants.

The first specific objective was to elucidate the mechanisms that control the adsorption of NH_4^+-N on biochar. For this purpose the effect of biochar ashes on NH_4^+-N adsorption was tested under controlled pH conditions and at different temperatures. Ammonium was selected instead of nitrate because: i) it is the main form of N adsorbed by biochar, since biochar is mainly characterized by the presence of negative charges (e.g. Taghizadeh-Toosi et al., 2012); ii) it is the first mineral N form produced from soil organic matter mineralization; iii) it is the first form of N derived from urea degradation and urea is the N fertilizer most used worldwide (Glibert et al., 2005); iv) it is the substrate used by nitrifying bacteria for the production of nitrate. This research aims to understand if the cation exchange capacity of biochar or its surface acidic groups are useful indicator to predict the biochar NH_4^+-N adsorption potential (Chapter 2).

A second scope of this research was to test if the N use efficiency of plant is affected by the enrichment of biochar with N fertilizer. The adsorption of NH_4^+-N fertilizer in a suspension with BC was compared with the conventional practice of applying N fertiliser directly on substrate. The underlying hypothesis was that the adsorption of NH_4^+ ions could negatively affect the N availability for plants, by subtracting mineral N to the cultivation system. Moreover biochar was tested as substitute of the most commonly used natural, but not eco-friendly, pot growing substrates: peat, perlite and zeolite. If biochar demonstrated to be suitable as amendment for soilless substrate it might contribute to the conservation of non-renewable peatland ecosystems, which are largely recognized for their role in biodiversity and C conservation (Charman, 2009).

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CHAPTER 2

Ammonium sorption onto *Eucalyptus* biochar: an isotherm study

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Abstract

Biochar (BC) has been proposed as sorbent of mineral nutrients, pollutants and organic molecules in soil and atmosphere environment, nevertheless the mechanisms underlying these phenomena remain still unclear. The main objective of this research was to investigate mechanisms involved on chemical interactions between BC produced from eucalyptus wood chips (Euc-350) and ammonium (NH_4^+-N) ions dissolved in aqueous solution. Adsorption isotherm curves of NH_4^+-N on (i) untreated biochar (designated 'original', O) or on biochar treated in diluted mineral acid (designated 'acid', A), were measured in a not titrated and in titrated (pH 7) solution. Moreover, in the latter case, the effect of temperature on adsorption of NH_4^+-N onto Euc-350 BC was investigated. The results demonstrated that the light acid treatment significantly affected the adsorption capability of NH_4^+-N onto Euc-350 in absence of titration. However, when both the liquid and the solid phase were titrated at pH 7, any significant difference was observed between A and O biochars, thus letting understand that the presence of minerals on BC surface did not affect the NH_4^+-N adsorption onto Euc-350. The temperature did not affect the adsorption parameters. The model that best describe the adsorption process is the Freundlich equation ($R^2 = 0.99$) The maximum measured amount of NH_4^+-N adsorption onto Euc-350 (1675 mmol kg^{-1}) greatly exceeds the total number of acidic functional group on biochar surface (635 mmol kg^{-1}) at pH 7. The revealed adsorption of NH_4^+-N onto BC may explain literature observations about the reduction in the soil of mineral N-pool accompanied by an increase in plant N-uptake when BC is added into the soil.

Keywords: biochar, ammonium, adsorption isotherm, pH, Freundlich.

2.1 Introduction

Nitrogen (N) is a key input in agriculture. Since the 20th century onwards, the wide use in agriculture of relatively cheap synthetic N fertilizer has made an unprecedented contribution to increasing food and feed production. Nevertheless only 30-50% of applied N fertilizer is taken up by crops (Smil 1999), while the remaining fraction is immobilized in organic compounds or lost to ground water and to atmosphere. Optimising the nitrogen use efficiency (NUE) is an absolute requirement for sustaining future population growth and reducing the environmental impact of agriculture.

Biochar is a carbon-rich solid co-product derived from the thermal-chemical transformation of biomass under complete or partial exclusion of oxygen (Lehmann et al., 2007). The interest in the use of biochar (charcoal) as a soil amendment has been increasing exponentially in the last decade. Biochar applied to the soil as a conditioner is recalcitrant to microbial degradation and leads to net removal of carbon from the atmosphere (Lehmann et al., 2007). As a soil additive along with organic and inorganic fertilizers, biochar has been reported to significantly improve soil texture and water holding capacity, nutrient availability to plants and plant productivity (e.g. Glaser, Lehmann, Zech, 2002). Evidence suggests that some of these positive effects of biochar may be strongly related to its interaction with the N cycle in soil. A significant and positive interaction of biochar with N fertiliser was observed by Chan et al. (2007), with higher yield observed with increasing rates of biochar application in presence of N fertiliser. Van Zwieten et al. (2010) demonstrated that the application of biochar could help increase NUE thus reducing the use of N fertiliser. Others also observed an increased NUE in presence of biochar (e.g. Kammann et al., 2011) and reduced NO_3^- (Ventura et al., 2012) or NH_4^+ (Ding et al., 2010) leaching. Moreover, additions of biochar resulted in reduced emissions of N_2O (Cayuela et al., 2013) from soil and NH_3 from compost (Steiner et al., 2010).

Despite numerous studies reported positive effects of biochar on the N cycle only few studies, almost all published in the last two years, investigated the mechanisms underlying the interactions between biochar and N. These mechanisms could be generally ascribed to two main groups: physical-chemical and biological interactions (see Fig. 2.1). The latter group includes N fixation by microorganisms and altered in the presence of biochar (Rondon et al., 2007), and microbiallymediated ammonification and nitrification in forest soils (DeLuca et al., 2006) and arable soils (Nelissen et al., 2012). Chemical interactions between biochar and the various N species include: adsorption of cationic and anionic species to relevant exchange sites on biochar surfaces (Steiner et al., 2008), and the reaction of ammonia at ambient conditions with surface oxygen groups leading to the formation of amines and amides (Seredych and Bandosz, 2007; Spokas, Novak, Venterea, 2012). Physical mechanisms include the entrapment of N in biochar pores (Clough et al., 2013) and a major N retention due to reduced water percolation after biochar addition to the soil (Major et al., 2009).

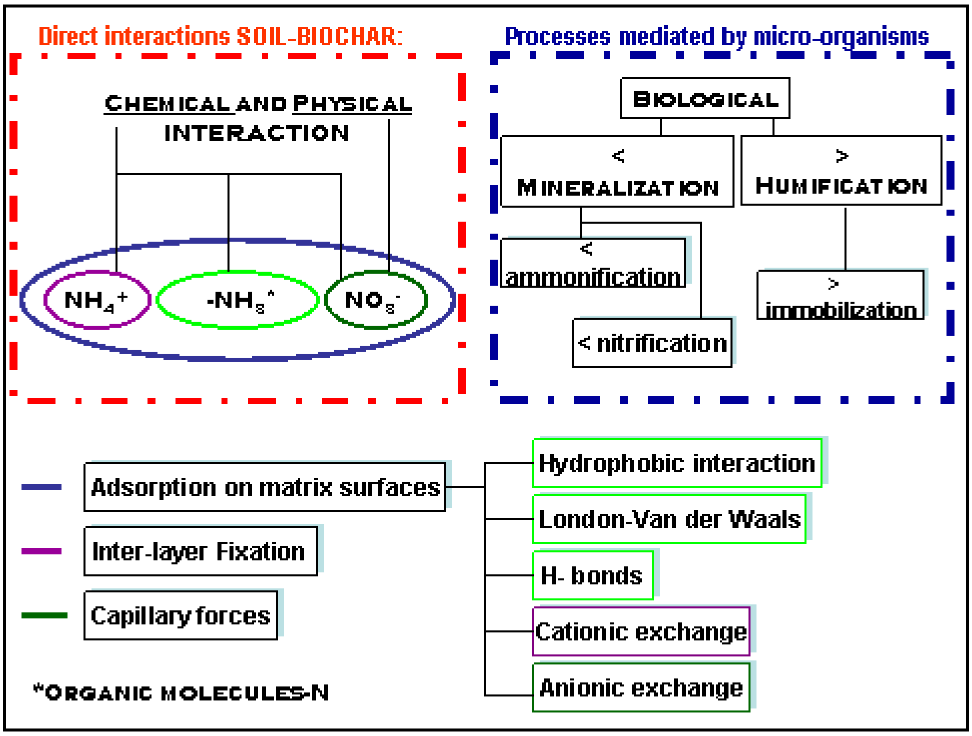

Figure 2. 1 Potential mechanism of interaction between biochar and nitrogen.

This work aims to focus on biochar and N chemical interactions, particularly referring to ammonium-nitrogen (NH_4^+-N) adsorption onto biochar. The chemical adsorption of NH_4^+-N (Hollister, Bisogni, Lehmann, 2013) or of NH_3-N (Asada et al. 2002; Yiobe et al. 2004) onto charcoal were found to be strictly dependent on the pyrolysis temperature at which it was produced, with higher adsorption at lower pyrolysis temperatures for a given feedstock. The lower the production temperature, the greater the number of acidic functional groups, as carboxylic groups, on charcoal surfaces (Mukherjee, Zimmerman, Harris, 2011). Similar results were obtained by Taghizadeh-Toosi et al. (2012), who demonstrated that, besides being strongly related to the biochar surface acidity (r^2 = 0.74), NH_3 adsorption onto biochar was yet more strongly related to the pH values of the biochar-water suspension (r^2 = 0.92). Indeed, the cation exchange capacity (CEC) of biochar is generally much higher in biochars produced at lower pyrolysis temperature (Mukherjee, Zimmerman, Harris, 2011). Conversely, Yao et al. (2012) did not found any trend in NH_4^+-N adsorption with increasing the pyrolysis temperature, although the pH of the compared biochars changed between 5.2 and 9.1. This fact, nevertheless, could be easily expected since they tested adsorption at only one single very low concentration of NH_4^+-N (10 mg L^{-1}) without studying the full adsorption isotherms. Also Hale et al. (2013) measured the sorption of NH_4^+-N onto biochar considering only a very low concentration range of 0.1 mg L^{-1} up to 50 mg L^{-1}. This may be one reason they did not find any difference in NH_4^+-N adsorption as a function of biochar feedstock. It is largely known that low adsorbate concentrations correspond high ratios between the amount adsorbed per unit weight of adsorbant and the adsorbate concentration in the liquid phase, leading to difficulties in understanding which biochar has a higher adsorption capacity than another. The low adsorbate concentration range is generally important for examining adsorption affinity of the adsorbent for the adsorbate, rather than adsorption capacity.

In order to evaluate the capability of a solid matrix to adsorb adsorbate molecules from aqueous solution, higher concentrations of NH_4^+-N must be used. For example, it was observed that the feedstock significantly influenced NH_4^+-N adsorption onto biochar when the initial NH_4^+-N concentration ranged from 1 up to 10,000 mg L^{-1} (Hollister, Bisogni, Lehmann, 2013). Nevertheless the findings reported in this last study were of limited use since it compared adsorption on biochars, washed and unwashed, characterized by very different pH values, ranging between 5.9 and 6.9 (Oak biochar) and 7.7 and 9.06 (Corn biochar). Thus the reported differences could be derived not from the nature of the biochar feedstock, but from the different pH at which the experiments were performed, because this strongly influences the protonation of acidic functional group in the biochars.

Only a few studies have investigated the release and consequent availability of the adsorbed NH_3 or NH_4^+ and, furthermore, reported contrasting results. In fact, while according to Taghizadeh-Toosi et al.(2011) the NH_3 adsorbed onto biochar (on average 6 mg g^{-1} of biochar) was plant available, Saleh et al. (2012) found that the adsorption of NH_4^+-N onto peanut hull biochar, produced at 450°C, was irreversible. However, Saleh et al. (2012) reported adsorption of 5g NH_4^+-N per g biochar, and according to their paper, worked at a pH of 9.3, which is more than the pKa of NH_4/NH_3. Thus, their results probably are related to losses of N to gas phase ammonia rather than irreversible adsorption. This experimental artefact may be common in other studies where pH was not carefully controlled, for example Hollister et al. (2013) and Hale et al. (2013).

Eucalyptus is one of the most valuable and widely planted hardwoods in the world with 20 million ha in 90 countries, in tropical, subtropical and temperate regions (Forrester and Smith, 2012). *Eucalyptus* spp. are largely appreciated for their i) fast growth, with rotation period shorter than 5 years in tropical areas; ii) high yields, as high as 70 $m^3ha^{-1}yr^{-1}$; iii) tolerance of harsh environments involving many effective adaptations: indeterminate growth, coppicing, drought, fire, insect resistance, and tolerance of soil acidity and low fertility. *Eucalyptus* wood is renowned for its high density that makes it suitable for sawn wood, pulp and paper manufacturing, fuel and charcoal production (Rockwood et al., 2008).

The main objective of this work was to examine the adsorption of NH_4^+-N onto Eucalyptus biochar under controlled pH conditions and at different temperatures, with the goal to elucidate the mechanisms that control its adsorption. To this end, we also tested the effect of biochar minerals on NH_4^+-N adsorption.

2.2 Materials and methods

2.2.1 Biochar production, characterization and rinsing treatment

Biochars from Eucalyptus (*Eucalyptus erythrocorys* F. Muell.) wood chips were produced by pyrolysis at temperature of 350 °C, 450 °C and 600 °C and then crushed to particle size < 500 μm. All biochars were characterized for their CEC at pH 7 according to Cohen and Graber (2013). The biochar produced at 350 °C (Euc-350) was, among all, the one with the highest CEC value at pH 7 and for this reason it was chosen to perform the present sorption study.

Subsamples of Euc-350 were dried at 40°C for at least 48 hours and analyzed for total C and N content using a CN Elemental Analyzer (NA-1500 series 2, Carlo Erba

Instruments, Milan, Italy). Also H, N, O and S contents of Euc-350 were measured. Total contents of Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr and Zn in the extracts were determined by an ICP-OES (Varian Inc., Vista MPX) on biochar subsamples oven dried at 105°C for at least 48 h and acid-digested with a microwave unit (CEM, MARSXpress) according to the EPA 3051A method (USEPA, 1995). After mineralization, the solutions were filtered (0.45 um PTFE), diluted and analyzed. Scandium was used as the internal standard. The total acidity of Euc-350 was determined by Boehm titration, after pre-treating the Euc-350 samples according to Tsechansky and Graber (2013). Total acidity is expressed as sum of carboxylic acid groups, lactonic acid groups and phenolic acid groups on carbon surface per unit weight of dried biochar prior to pre-treatment and given in mmol kg^{-1}. All characterization analyses were conducted in triplicate. The CEC of Euc-350 was measured at different pH values, according to Cohen and Graber (2013). pH of biochar suspensions was measured in distilled water in a 1:10 solid: liquid ratio, using an ‘CyberScan pH 10’ (Eutech Instruments).

A portion of Euc-350 was acidified with dilute mineral acid (0.01 M HCl) at room temperature to remove basic minerals present on surface, by stirring in a 1:50 Euc-350: liquid ratio for 48 hours. After the stirring, Euc-350 was vacuum filtered using a Whatman n°1, then rinsed several times with distilled water (DW) in order to remove the excess acid (Silber, Levkovitch and Graber, 2010), which was confirmed by testing for Cl using $AgNO_3$. It was reported that a mild acidification treatment did not significantly change the biochar surface charge but only simulates the effect of mineral leaching from biochar (Silber, Levkovitch and Graber, 2010). The acid treated biochar (designated ‘Acid’, A) and the not treated biochar (designated ‘original’, O) were dried in an oven at 105°C, until they reach a stable weight, and stored in a closed glass container.

2.2.2 Preliminary experiment: adsorption kinetics

The kinetics of NH_4^+-N adsorption onto A and O biochar from aqueous solution was investigated to assess the time necessary to reach an equilibrium. The liquid phase consisted of an aqueous solution of ammonium chloride (NH_4Cl) at an NH_4^+-N concentration of 100 mg L^{-1}. For this preliminary experiment, pH was not controlled. Biochar (1 g) was weighed in a 15 ml polypropylene (PP) test tube and 10 ml (V_0) of NH_4Cl solution were added (1:10 solid:liquid ratio). Finally the test tube was hermetically sealed. Each sample was done in triplicate, with two negative controls: (i) NH_4 solution, no biochar; and (ii) biochar, 0.1 M NaCl in place of NH_4 solution, in triplicate. The first control aimed to evaluate possible NH_4^+-N losses from the experimental system, while the second control aimed to assess the possible release of NH_4^+-N from biochar. Samples were shaken with a mechanical shaker at room temperature for six different intervals of time (1- 4 – 8 – 24 – 48 - 72 hours). After shaking, each sample was centrifuged for 20’ at 5,000 rpm, the supernatant was collected and filtered using a 0.45 μm PVDF filter in order to avoid the presence of biochar particles. The concentration of NH_4^+-N in the liquid phase was measured colorimetrically following the protocol described by Willis et al. (1996) using a UV-Vis spectrophotometer ThermoScientific (Genesys 10 UV). The amount of NH_4^+-N adsorbed per unit weight of adsorbent (q_e) was calculated as the difference between the initial (C_i) and the final (C_f) NH_4^+-N concentration in the liquid phase, multiplied by the volume of the NH_4Cl solution (V_0) and divided by the weight of the biochar sample (W_b) :

$$q_e = (C_i - C_f)*V_0 / W_b \quad (1)$$

Results are expressed as mmol kg^{-1} of NH_4^+-N adsorbed onto biochar.

2.2.3 Equilibrium adsorption isotherms with pH-adjusted biochars at 20°C, 40°C and 55°C

In order to avoid pH effect on adsorption isotherm curves, O and A biochars were titrated to pH 7 at a solid:liquid ratio of 1:20 (100g and 2 L). The titrations were carried out at 25°C using a Mettler Toledo DL – 50 automatic titrator (Columbus, OH, USA) under continuous magnetic stirring. To O and A biochars, 27 ml of 0.1 M HCl and 44.23 ml of 0.1 M NaOH respectively, were added during the titrations, to bring the solution in equilibrium with the biochar to pH 7. By pre-equilibrating to pH 7, we were assured that the system was at least 2 pH units below the NH_4/NH_3 pKa, ensuring that NH_4^+ is the dominant species, and minimizing potential losses as NH_3 gas. After titration, the solid and the liquid phases were separated by sequential filtration using Whatman 1 paper, and the biochar was wrapped and stored under refrigeration at 4°C. The supernatant was further filtered through Whatman 2 paper and finally, with a 0.22 μm sterile single-use filtration system (Corning Filter System Polystyrene, sterile). The supernatant was stored in the sterile closed container at +4°C and subsequently used to prepare the NH_4Cl solutions for the adsorption experiments. Eleven concentrations of N-NH_4Cl solution were prepared: 5, 20, 50, 70, 100, 700, 1400, 2100, 4200, 8400, and 17500 mg L^{-1}.

Pre-titrated moist biochar (weight was pre-determined taking into account each time the measured moisture content), was added together with NH_4Cl solution to a 15 ml PP test tube, which was promptly hermetically sealed. To evaluate the moisture content, a sample of moist biochar was weighed, rinsed several times with DDW to eliminate salts, dried in the oven at 105°C until reaching a stable weight, and re-weighed. The solid: liquid ratios used were 1:10 for each solution concentration except for 8400 and 17500 mg L^{-1} in which a 1:5 solid: liquid ratio was used and for 5 mg L^{-1} concentration in which the solid: liquid ratio used was 1:100. To allow maximum level of adsorption, biochars sample and liquid phase remained in contact for 48 hours then the samples were analyzed as detailed before. The amount of NH_4^+-N adsorbed per unit weight of adsorbent (q_e) was calculated as the difference between the initial (C_i) and the equilibrium (C_e) NH_4^+-N concentration in the liquid phase, multiplied by the volume of the NH_4Cl solution (V_0) and divided by the weight of the biochar sample (W_b) :

$$q_e = (C_i - C_e) * V_0 / W_b \qquad (2)$$

Results are expressed as mmol kg^{-1} of NH_4^+-N adsorbed onto biochar.

In order to obtain insights in adsorption mechanisms, isotherms were measured at three different environmental temperatures (20, 40, and 55°C on a thermostated shaker, SI-600R, Lab. Companion). The choice of these temperature values was suggested by specific reasons: 20 degree was thought as room temperature, while 55 degree is essentially the highest temperature reached during the composting process.

Each concentration point was done in triplicate, with two negative controls: (i) NH_4 solution, no biochar; and (ii) biochar, 0.1 M NaCl in place of NH_4 solution, in triplicate. The first control aimed to evaluate possible NH_4^+-N losses from the experimental system, while the second control aimed to assess the possible release of NH_4^+-N from biochar. Each collected supernatant was analyzed for Na, Cl, pH and electrical conductivity (EC), used to calculate the ionic strength and NH_4^+ activity in the aqueous phase at the equilibrium using Visual Minteq (ver. 3.0, online available). These calculated parameters, together with the isotherm parameter (q_e and C_e) were used to calculate the sorption enthalpies (isosteric heats). Enthalpy changes associated with sorption process were estimated according to the methodology described by Huang and Weber (1997).

2.2.4 The Langmuir and Freundlich models: calculation methods

Langmuir and Freundlich models were used to fit the experimental data and the equations used were, respectively

$q_{e} = (Q_{max} * b * C_e) / (1+ bC_e)$ (3)

$q_{e} = K_f * C_e^{(n)}$ (4)

where q_e is the amount of the NH_4^+-N adsorbed per unit weight of biochar (mmol kg^{-1}), C_e is the equilibrium concentration in solution (mmol L^{-1}), Q_{max} is the maximum adsorption capacity (mmol kg^{-1}) and *b* is the constant related to the affinity. K_f and *n* are empirical constants which can be related to adsorption phenomena. Q_{max} and *b* can be determined from the linear plot of C_e/ q_e versus C_e, where $1/Q_{max}$ is the intercept and 1/*b* is the slope of the linear plot. Equation (4) can be transformed by applying the log form:

$\log q_e = \log K_f + n* \log C_e$ (5)

where K_f and *n*, which units of measure depend on each other, can be determined from the linear plot of (5). K_f is the amount of NH_4^+-N adsorbed onto biochar when C_e is equal to 1, and *n* is a parameter often taken to indicate the relative affinity of the adsorbent for the adsorbate as a function of Ce. Freundlich K_f parameters are not comparable unless the *n* parameters are the same (Bowman et al., 1982). In fact, the units of K_f follow directly from a dimensional analysis of rearranged eq. (4) and, since here the units of q_e are mmol kg^{-1} and for C_e are mmol L^{-1}, then the units of K_f will be: K_f (units) = (mmol kg^{-1})/ (mmol $L^{-1})^n$ = $mmol^{(1-n)}$ kg^{-1} L^n (Bowman, 1982). A great variety of units were used for the two adsorption variables q_e and C_e, which in turn determine the units of K_f. Moreover, authors often did not indicate that K_f has units, thereby resulting in the mistaken belief by some that K_f is an unitless constant. For these and for other interesting reasons discussed by Bowman (1982) is not possible to compare Freundlich K_f parameters obtained by different studies.

2.2.5 Statistical analyses

Results of the kinetics study (see par.2.3.2) were analyzed by two way analysis of variance (ANOVA) which considered the acidic treatment and the time of contact as main factors in a fully randomized design. Effect of acidic treatment and temperature on NH_4^+-N adsorption onto biochar at 20, 40 and 55 °C, were tested via two-way ANOVA in a fully randomized design. An assumption of ANOVA is the normal distribution of the data. To test the homogeneity of variances of our data Bartlett's test was carried out before performing each ANOVA test ($P>0.05$). All statistical analysis were carried out by using CoStat (version 6.204, CoHort Software). Significant differences among treatment groups were determined according to Tukey test ($P<0.001$).

2.3 Results

2.3.1 Biochar characterization

The CEC measured at pH 7 of biochars produced at 350 °C, 450 °C and 600 °C were of 496.8 mmol kg^{-1}, 343.2 mmol kg^{-1} and 193.1 mmol kg^{-1} respectively. Euc-350 was chosen as the best biochar to conduct the present study because of its high CEC.

The elemental composition and the main physical-chemical properties of the Euc-350 biochar are shown in Table 2.1, while the mineral nutrient content, expressed in mg kg^{-1}, is shown in Table 2.2. A and O biochar gave different suspension pH values (6.6 and 7.8, respectively), demonstrating that alkaline minerals are removed upon the dilute HCl treatment, as intended. The CEC values of EUC-350 (Fig. 2.2) increased as with increasing pH, resulting of 404 mmol kg^{-1} and 498 mmol kg^{-1} at pH 6.6 and 7.3, respectively. This is in accordance with previous findings that CEC of biochar increases with increasing pH (Silber et al., 2010). The results of the modified Bohem titration, conducted after pre-treating dried weighed biochar subsamples firstly with aqueous NaOH to solubilize and remove organic and inorganic acidic contaminants, and then with aqueous HCl to remove basic species and protonate acid sites on the carbon, should be regarded as maximum possible number of carbon surface functional groups (*ncsf*) per unit weight of un-treated biochar (Tsechansky and Graber 2013), and here was close to the CEC measured at pH 7 for Euc-350 biochar (635 mmol kg^{-1}, Table 2.3).

Table 2. 1 Physical-chemical characteristics of Euc-350 biochar. Results are average and standard error of three replicates, with the exception of those designated with *, where only one replicate was measured.

N (% on D.W.)	1.3 ± 0.19
C (% on D.W.)	69.3 ± 3.46
H (% on D.W.)	3.1 ± 0.12
O (% on D.W.)	17.7 ± 0.88
Ash (% on D.W.)	12*
S_{BET} (m^2g^{-1})	4*
CEC[1] - pH 6.6 (mmol kg^{-1})	404 ± 28
CEC[1] - pH 7.3 (mmol kg^{-1})	497 ± 2

[1] – Cation Exchange Capacity. Average of two replicates is given

Table 2. 2 Mineral nutrient content of Euc-350 biochar. Results are average and standard error of three replicates.

Mineral Nutrient	**Content (% on D.W.)**
Ca	3.57 ± 0.748
K	0.96 ± 0.163
Fe	0.61 ± 0.025
Mg	0.27 ± 0.080
P	0.10 ± 0.003
Na	0.10 ± 0.048
Al	0.03 ± 0.007
Zn	0.01 ± 0.001
Mn	0.01 ± 0.000

Table 2. 3 Total number of carbon surface functional groups (*ncsf*) on Euc-350 (mmol kg^{-1}) at pH 7.

	Total Acidity	**Carboxylic**	**Lactonic**	**Phenolic**
EUC-350	635 ± 8	116 ± 7	246 ± 10	273 ± 11

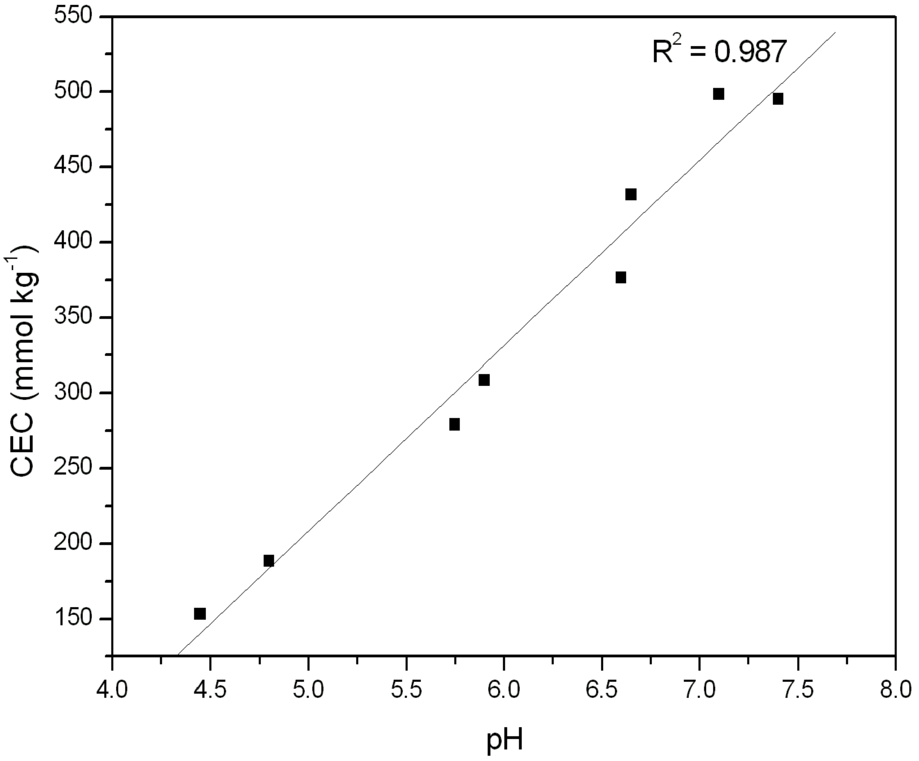

Figure 2. 2 CEC of Euc-350 biochar at varying the pH conditions

2.3.2 Kinetics study

Both the acidic treatment (A, O) and the time of contact between biochar and the liquid phase (1 – 4 – 8 – 24 – 48 - 72 hours) significantly affected the adsorption of NH_4^+-N onto biochar ($P < 0.001$), but no significant effect derived from the interaction between the two considered factors. Once NH_4Cl solution was added to the biochar, concentrations of NH_4^+-N in the liquid phase immediately decreased (Fig. 2.3). Already after the first hour of contact, A was significantly more effective than O ($P<0.001$) in adsorbing NH_4^+-N (0.53 mg g^{-1} vs 0.31 mg g^{-1}; Table 2.4). Subsequently, the concentration of NH_4^+-N in the aqueous solution continued to gradually decrease with increasing the contact time, until sorption equilibrium was reached. The sorption kinetics was quite similar, and A and O biochars reached equilibrium after 8 hours by adsorbing, respectively, 0.63 mg g^{-1} and 0.40 mg g^{-1} of NH_4^+-N. Analysis of the control samples showed that no sorption occurred on the walls of the test tube, nor were there losses due to volatilization. NH_4^+-N released from A and O biochars in the negative control was insignificant.

Table 2. 4 Preliminary kinetic study. Amount of $NH4^+$-N adsorbed onto biochar after the 1st hour of contact (q_1) and at equilibrium (q_e) between biochar and liquid phase.

	q_1 (mg g^{-1})	q_1 (mmol kg^{-1})	q_e (mg g^{-1})	q_e (mmol kg^{-1})
O	0.31	22.13	0.40	28.92
A	0.53	37.84	0.63	44.95

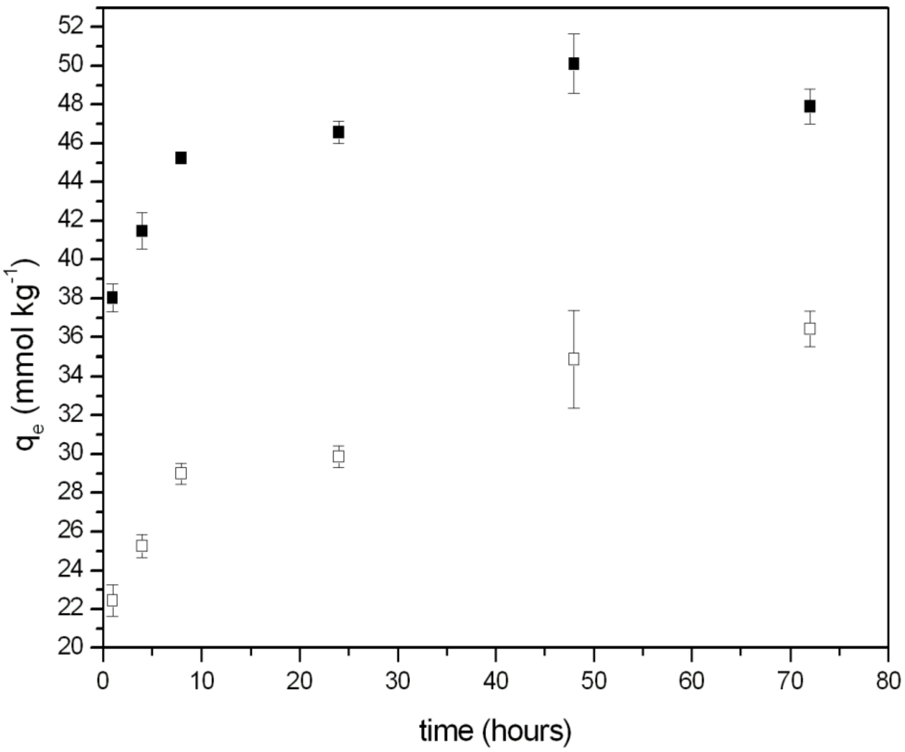

Figure 2. 3 Kinetic study. Effect of contact time on NH_4^+-N adsorption capacities by Euc-350 biochar, treated with acid (■) and original (□). Means ± S.E. (n=3)

2.3.3 Effect of minerals and temperature on NH_4^+-N adsorption onto pH-adjusted biochar

The amount of NH_4^+-N adsorbed onto biochar (q_e) increased continuously with an increase in solution concentration, while the ratio between q_e and C_e decreased as C_e increased. All curves were similar in shape, typical of nonlinear isotherms (Fig. 2.4). No clear adsorption plateau was reached. No significant effect derived from the acidic treatment, meaning that the presence of minerals localized on biochar surface did not affect the adsorption of NH_4^+-N onto biochar. Moreover, temperature at which adsorption was carried out had no effect on the adsorption isotherms (Fig. 2.4). The isosteric heat of sorption calculated at 1, 10, 100 and 1000 mmol kg^{-1} of C_e (Fig. 2.5) demonstrates a slope of zero versus temperature, thus allowing the exclusion of possible exothermic reactions which are typical of physisorption mechanism (Huang and Weber, 1997). The NH_4^+-N activity coefficient measured in the liquid phase was also not affected either by temperature or by the acidic treatment (0.76 ± 0.02). The average equilibrium pH value measured in the samples at the end of the experiment was 7.4 ± 0.6 s.d.

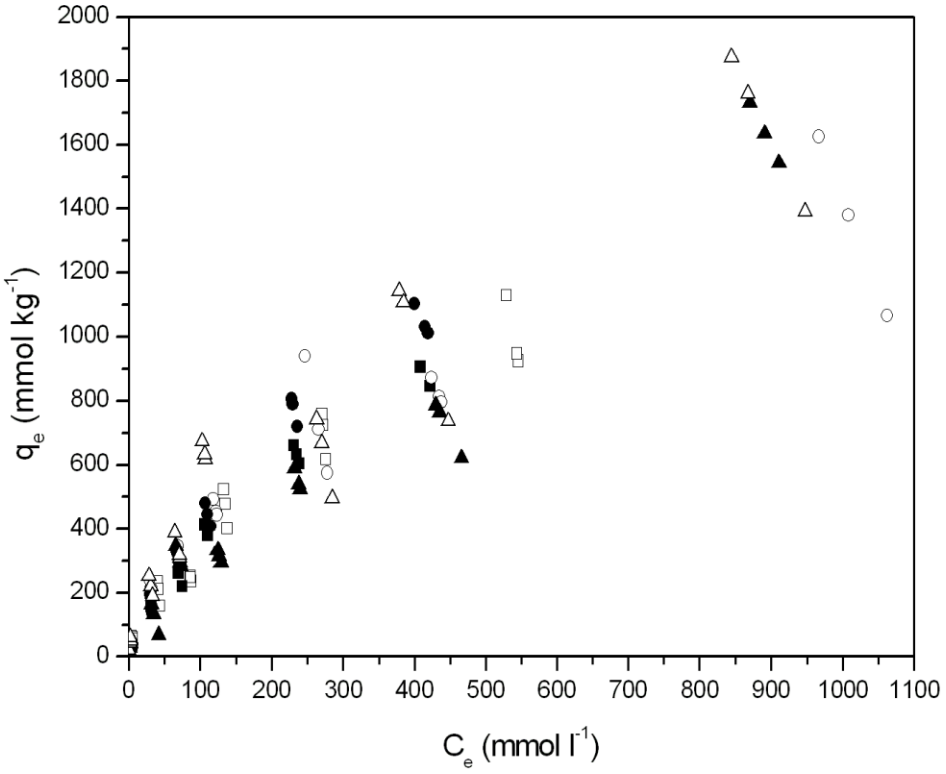

Figure 2. 4 Effect of temperature on the adsorption effectiveness at pH 7 of Euc-350 for NH_4^+-N treated with acid: 20°C (■), 40°C (●), 55°C (▲); original: 20°C (□), 40°C (○), 55°C (Δ)

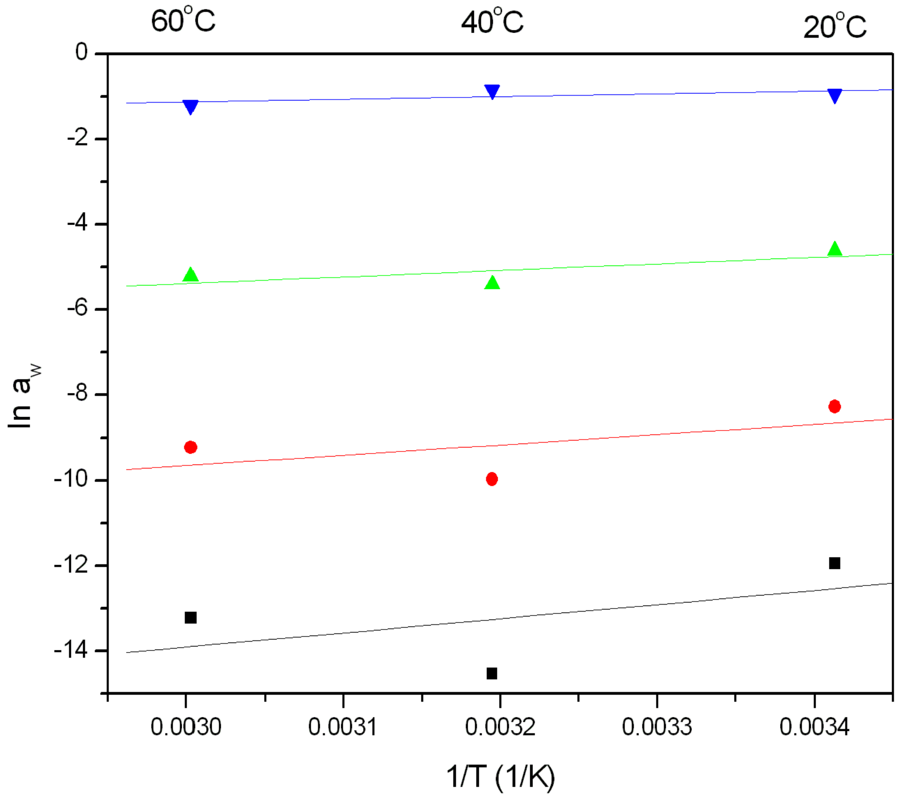

Figure 2. 5 Isosteric energy of adsorption at 1 (■), 10 (●), 100 (▲), 1000 (▼) mmol kg^{-1} of sorbed concentration (qe). On x axis the temperature is expressed in Kelvin degree.

2.3.4 The lack of plateau and the suitability of the Freundlich model

The Langmuir model does not give a very good fit to the adsorption data (Table 2.6). There it can be seen that a hypothetical maximum adsorption capacity for NH_4^+-N was estimated, according with equation 3, to be 1614 and 1563 mmol kg^{-1}, for A and O biochars respectively. Since we did not reached these levels of adsorption at our initially chosen highest Ci of 8400 mg L^{-1}, we added an additional C_i concentration point, at 17500 mg L^{-1}. Unfortunately we did not have enough material to carry out the experiment at all combinations of temperature and biochar, thus the 17500 mg L^{-1} point was tested only with O biochar at 40°C and 60 °C and with A biochar at 60 °C. These last data were analyzed using Student t-test. At the concentration of 17500 mg L^{-1}, the adsorbed amount of NH_4^+-N onto biochar was 1675 and 1637 mmol kg^{-1} respectively for O and A biochars at 60°C (Table 2.5) while it was of 1357 mmol kg^{-1} for O biochars at 40°C. The three series of values were not statistically different. The expected Langmuir threshold, was overcome. Moreover, the maximum amount of NH_4^+-N adsorbed onto biochar highly exceeded the CEC measured at pH 6.6 and 7.3 (Fig. 2.2), as well as the total number of acidic functional groups (Table 2.3). The lack of plateau and poor fitting results demonstrate the unsuitability of the Langmuir model for describing NH_4^+-N adsorption onto Euc-350 biochar.

As a result, the data was also fitted by the Freundlich model. The adsorption constant and correlation coefficients for the Freundlich isotherms are given in Table 2.6. Correlation coefficients suggested that the Freundlich model ($R^2 = 0.99$, Fig 2.6), fit the data better than the Langmuir model ($0.74 < R^2 < 0.93$). The *n* constant estimated by fitting data with the Freundlich model, resulted lower than 1, lead us defining the obtained adsorption isotherms as characteristic of type L isotherms, with site-specific interactions between NH_4^+-N and biochar. While some of these sites may certainly be exchange sites, it is apparent that there is yet an additional type of interaction for NH_4^+-N, which needs additional study to decipher.

Table 2. 5 Maximum amount of NH_4^+-N adsorbed onto Euc-350 biochar measured in this experiment.

	C_e (mmol l^{-1})	**q_e (mmol kg^{-1})**	**q_e (mg g^{-1})**	**SE (mg g^{-1}, n=3)**
O	887	1675	23.5	2.04
A	891	1637	23.0	0.77

Table 2. 6 Constants and correlation coefficients of Langmuir and Freundlich model for NH_4^+-N adsorption onto Euc-350 biochar

		Langmuir model			**Freundlich model**		
Treatment	**Temperature (°C)**	**q_{max}**	**b**	**R^2**	**K_f**	**n**	**R^2**
O	**20**	1053	0.0095	0.88	25.30	0.57	0.99
A	**20**	935	0.0108	0.91	19.39	0.62	0.99
O	**40**	1314	0.0084	0.93	29.16	0.56	0.99
A	**40**	1139	0.0111	0.91	23.13	0.63	0.99
O	**60**	1563	0.0075	0.87	33.02	0.57	0.99
A	**60**	1614	0.0039	0.74	25.02	0.57	0.99

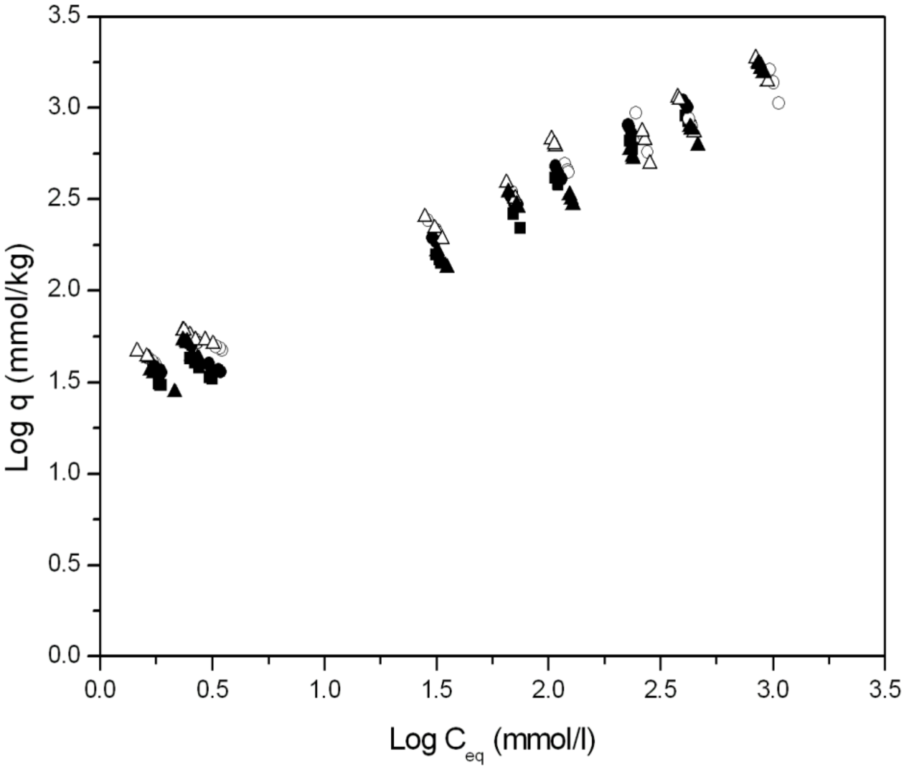

Figure 2. 6 Adsorption isotherm of ammonium ions onto Euc-350 biochar fitted by Freundlich model. Biochar treated with acid (▲) and original biochar (Δ)

2.4 Discussion

The present study was carried out at fixed pH conditions, as only few other adsorption studies of NH_4^+-N on biochar did before. It is largely known that pH is one of the most important parameters affecting ammonium cation adsorption (Bandosz, 2006), through the effect of pH on adsorbent surface charge and ionization, as well as its influence on the degree of ionization and speciation of the adsorbate (Regmi at al., 2012). Moreover, controlling pH well below the pKa NH_3/NH_4 is essential in order to avoid ammonia (NH_3) losses. Maintaining a fixed pH condition when evaluating the actual capability of each biochar to adsorb ammonium from aqueous solution, is essential in order to enable meaningful comparisons and understanding of mechanisms. Moreover, adopting this methodology allow to characterize and compare biochars produced with different pyrolysis plants, from different feedstocks and at different temperatures, with the final objective to determine the biochar best adapted to retain NH_4^+-N. This study demonstrated that the adsorption of NH_4^+-N is independent from the presence of minerals on biochar surface. Also, Hale et al. (2013) found that a rinsing treatment of biochar with Millipore water had no effect on the adsorption of NH_4^+-N onto biochar. On the other hand Hollister et al. (2013), which considered a larger range of C_i (10 - 10,000 mg L^{-1}) compared to Hale et al. (2013), found that a pre-rinsing treatment made with cold water decreased the sorptive capacity of Oak and Corn cob biochars for NH_4^+-N. However, the changes in adsorption capacity found by these authors could derive from changes in pH which followed rinsing: from 4.32 to 5.88 in case of Oak biochar and from 7.95 to 7.78 in case of Corn biochar. Neither Hale et al. (2013) nor Hollister et al. (2013) controlled the experimental pH; this creates confusion for understanding whether changes in adsorption upon rinsing derives from the treatment itself or from pH-induced differences in sorption capacity.

In the present study, no effect of adsorption temperature (20, 40, 55°C) on the uptake of NH_4^+-N onto Euc-350 biochar was found. This finding supports the hypothesis that physisorption mechanisms are not involved in NH_4^+-N adsorption onto Euc-350. The lack of apparent physisorption mechanisms was demonstrated also by calculating the isosteric heats of adsorption, which demonstrated a slope of zero (Fig. 2.5). The absence of temperature dependence was already shown for NH_4^+-N adsorption onto woody charcoal at temperatures of 5 and 20°C (Iyobe et al., 2004), nevertheless this is the first study, to our knowledge, that investigated how NH_4^+-N adsorption onto biochar may be affected at so high temperatures, usually occurring, for example, during the composting process (55°C). Our results supported the possibility that adding biochar into composting system could increase the quality of the final product by trapping NH_4^+-N and reducing N losses in forms of volatilized ammonia or leached nitrate (Steiner et al., 2010).

The CEC of the biochar is generally given as the rationale for the NH_4^+-N adsorption capability onto biochar (Clough et al., 2013). Nevertheless, the results of this study show that Euc-350 adsorbs NH_4^+-N cation at least until 1600 mmol kg^{-1}, without reaching saturation. This level of adsorption at pH 7 greatly exceeds the number of carbon surface functional groups measured by the Bohem Titration (635 mmol kg^{-1}), as well as the measured CEC (Fig. 2.2), leading to the conclusion that other kinds of adsorption phenomena, in addition to cation exchange, may be associated with NH_4^+-N adsorption onto biochar. This may be hydrogen bonding, covalent bonding or ligand exchange (Mukherjee et al., 2011). These possibilities need more work in order to decipher their role, if any, in NH_4^+-N adsorption.

The Langmuir model was not appropriate for describing the mechanisms involved in NH_4^+-N adsorption onto Euc-350, because of the lack of plateau, which resulted in a poor model fit ($0.74 < R^2 < 0.93$). Conversely, the Freundlich model well fitted our data ($R^2= 0.99$). The Langmuir model describes monolayer adsorption onto a single type of adsorption sites characterized by a single energy level, with no interactions between adsorbed molecules. The Freundlich may be envisioned as a collection of many Langmuir isotherms with an exponential distribution of specific site energies (Huang and Weber, 1997). It should be noted, that despite its widespread use, the Freundlich model is mathematically unable to give a linear part of the isotherm in the low concentration Henry's region.

Taking into account (i) the shape of the obtained adsorption isotherm ($n<1$), (ii) the lack of a temperature effect, (iii) an isosteric energy of zero, (iv) the adsorption which exceeded the CEC and *ncsf*, and (v) the lack of adsorption plateau, it is possible to speculate that the adsorption of NH_4^+-N onto biochar involves the co-existence of different specific chemical mechanisms and to exclude, instead, physical adsorption and nonspecific interactions. A portion of the total amount of NH_4^+-N adsorbed onto biochar could be explained by the contribute of the CEC and *ncsf*, while the contribution of yet undefined interactions, could explain the amount of NH_4^+-N adsorbed that exceed the CEC and *ncsf*.

The use of only a single biochar is a limit of this study, nevertheless it should be thought as representative of biochar produced by pyrolysing coppice wood at a relative low temperature (350°C). Further experiments are needed to confirm these results on other kinds of biochars, like Euc biochars produced at different temperatures or biochars made from different feedstocks. Moreover further analysis is desirable to elucidate the nature of all the chemical pathways involved in the relation between NH_4^+-N and biochar.

According with the results of this experiment, A and O biochar shown to potentially adsorb, at least, an amount of NH_4^+-N of 22.92 g kg^{-1} and 23.47 g kg^{-1} respectively, without reaching any plateau. It was demonstrated that biochar oxidation, which naturally occurs after burying biochar in the soil, leads to the development of carboxylic and phenolic functional groups and negative charges while decreasing the carbon content and the surface positive charge (Cheng et al., 2008). Hence it might be expected that, by over time, the contribution of CEC and *ncsf* to ammonium adsorption onto biochar would increase, thus improving the amendment effect of biochar itself and particularly its effect against leaching of N in the groundwater. Therefore the distribution of fresh biochar together with NH_4^+-N fertilisers could help in retaining the N in the surface layer in which roots are more developed, finally increasing the plant's nitrogen use efficiency (Steiner et al., 2007).

2.5 Conclusions

The present study observed the phenomena of NH_4^+-N adsorption onto Euc-350 biochar, by maintaining constant pH conditions between adsorbate and adorbant. It was demonstrated that Euc-350 biochar can adsorb ammonium ions through specific chemical mechanisms of interaction, independent of the environmental temperature at least up to 55° C, and independent of the presence of minerals on biochar surfaces. Ammonium adsorption largely exceed the CEC and the number of carbon surface functional groups of the Euc-350 biochar, thus leading to infer that there is an additional interaction mechanism involved besides cation exchange, for example, hydrogen bonds. The Freundlich model fit the experimental isotherm data here obtained better than the Langmuir model, which moreover should be excluded by the lack of a plateau in ammonium adsorption onto Euc-350.

In evaluating the capability of biochar to retain N before distributing it to the soil, numerous variables have to be considered, since, it is widely known, that biochar nature is not stable but is strictly dependant on the chemical and physical characteristics of the original biomass and on the physical conditions developed during the pyrolysis process (e.g. temperature, rate of temperature increase, feedstock). This study suggests that such pH controlled adsorption batch experiments will be useful in predicting the biochar capability to retain NH_4^+-N. This biochar characteristic must be taken into account in choosing suitable biochars for agronomic use, for instance, in agricultural sites in areas with a very shallow groundwater, or in composting material, with the intention of reducing risks of N leaching or of N volatilization losses. Since biochar is distributed in the top-layer of the soil, its capacity to retain N could result, in the long period, in a reduced needed for N fertilizers and in a more effective use of them.

2.6 Future works needed

1. Verifying lack of losses of NH_4^+-N from the experimental system.
2. Confirming NH_4^+-N adsorption results with other biochars and additional adsorption methodologies.
3. Testing adsorption of a cation of the same hydrated size that lacks the potential for H-bonding, to help decipher mechanisms controlling NH_4^+-N adsorption.
4. Measuring NH_4^+-N adsorption on biochar at a different pH.
5. Determining release of other cations (protons, Ca, Mg, Na, K) and anions (PO_4) during adsorption of NH_4^+-N.
6. Studying the release characteristics of adsorbed NH_4^+-N and its plant availability in lab, pot, and field studies.

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CHAPTER 3

The synergism between nitrogen fertilizer and biochar in the composition of soilless substrate

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Abstract

Nursery production is characterized by intense cropping system with high input of fertilizers and irrigation. A further concern is the high cost of soilless substrates, which often derive from limited and non-renewable sources, such as peat and perlite. The main objectives of this study were: 1) to evaluate the potential use of biochar (BC), a by-product of the pyrolysis, as possible growing media substrate in comparison with other common used amendments; 2) to assess if enriching BC with nitrogen (N) fertilizer prior to its application to soilless substrate could enhance its effectiveness. For these objectives a factorial experiment was established combining five different amendments with three methods of application of N fertilizer. A total of 17 treatments was replicated 5 times in a pot experiment (completely randomized design). The pots (1100 cm^3 volume) were filled with nutrient free quartz soil (sand) in order to avoid the effect of a natural soil on the balance of the mineral N added to the soil-amendment mixture. The five amendments were: peat (PT), perlite (PR), zeolite (ZL), biochar derived from fruit trees pruning wood (BC1) and biochar produced by pyrolysis of coppiced wood (BC2). The three N fertilization treatments were: no N addition (no_N_f); N-fertilizer solution (150 mg N pot^{-1}) added to the pure amendments before the addition to the pots' soils (amend_N_f), or the same N-fertilizer solution applied on the soil-substrate mixture of each pot (mix_ N_f). Plant response (*Lolium perenne L.*) was evaluated in terms of number of seedlings survived (% emergence), above- and below-ground biomass, nutrients content of above-ground biomass, N use efficiency (NUE = ratio of total dry biomass produced per N inputs) and water productivity (WP = total amount of dry biomass produced per H_2O consumed). Furthermore at the end of the experiment, the following soil parameters were measured: available P and enzymatic activities of Leucine-aminopeptidasi (Lap), alkaline (alkP) and acid phosphatase (acP).

All BCs demonstrated to be suitable substitute for commonly used peat in growing media compositions. The best BC was that derived from fruit pruning trees residues (BC1) because of its higher content of mineral nutrients. A positive interaction effect was observed for N fertilization combined with BC. The total biomass, WP and NUE were the most influenced parameters. The high performance of BC1 combined with N fertilizer, was sustained by significantly higher leaf content of P and K compared to the other substrates. The mix_N_f BC1 improved total biomass, WP and NUE compared to BC2 and peat, and resulted, meanwhile, significantly higher than BC1 amend_N_f in terms of total biomass.

Keywords: biochar; nitrogen; growing media; water productivity; peat; pyrolysis.

3.1 Introduction

The total volume of growing media used in the EU (hobby and professional) is estimated to be approximately 45 million m^3 (or 15 million tons) annually and the consumption of peat-based substrates is approximately 19 millions m^3 $year^{-1}$ (Joosten, 2005). The environmental and economical sustainability of the growing media used is one of the most important factors to take into account in evaluating the suitability of each growing media together with the performance in terms of crop production, water and nutrient-use efficiencies, biological stability.

Peat is one of the most used growing media components and it is largely appreciated for its high cation exchange capacity (CEC), total porosity and water holding capacity (WHC), low bulk density and pH (Hemstock et al., 2008). However, the extraction of peat from non-renewable peatland endangers their conservation (Charman, 2009). As a consequence, the price of peat is increasing also because it is affected by the energy costs of all the stages of the production process. All the reasons above have generated high interests in finding new potential substrates to replace peat (Carmona et al., 2012; Restrepo et al., 2013). Many studies have shown that mineral substrates such as zeolite (e.g. Ayan and Tufekcioglu, 2006) and perlite are possible peat substitutes. For instance, zeolite is known for its high WHC and CEC (Hedstrom, 2001) that allows high nutrients retention, particularly nitrogen (N) as ammonium (NH_4^+) or potassium (K). A new peat substitute candidate could be biochar (BC), a carbon-rich co-product of the pyrolysis of various biomasses. Biochar (charcoal) is a solid material obtained from the carbonization of biomass. In more technical terms, biochar is produced by thermal decomposition of organic material under limited supply of oxygen (O_2), and at relatively low temperatures (<700°C, http://www.biochar-international.org). The addition of charcoal in a basal substrate constituted of peat, soil, vermiculite, perlite and sand, was studied to evaluate the productivity of transplanted French marigold (*Tagetes patula*), melampodium (*Melampodium paludosum*), scarlet sage (*Salvia splendens*) and zinnia (*Zinnia linearis*). No effect of charcoal addition were observed on plant heights of French marigold or scarlet sage plants, while significant improvements were obtained in case of zinnia, in terms of survival rate, plant height and number of branches and in case of melampodium, in terms of plant height (Kadota and Niimi, 2004). Dumroese et al. (2011) evaluated some physical-chemical properties of substrates constituted of pure peat amended with increasing doses of pelleted biochar. They speculated that the most appropriate substrate for use in nurseries crop was constituted of 25 % of pelletted biochar and 75 % of peat. Nevertheless this assessment has been done without growing any plants. Transplanted plants of *Calathea rotundifolia* were grown in soilless substrates constituted of only peat, of a mix 1:1 peat:BC or of BC alone (Tian et al. 2012). This latter case, as expected, caused a high reduction in plant productivity. The peat 'amended' with BC demonstrated a significantly higher above-ground biomass production compared to peat alone, accompanied by a reduced media degradation and an increased pH. Recently Vaughn et al. (2013), assessed the substitution of peat with BC at 5%, 10% and 15%, obtaining an increase in plant height of tomato (*Solanum lycopersycum*) and French marigold (*Tagetes erecta* L.) but no effect on plants dry weight. No one of these studies compared peat and BC use as amendment in the composition of soilless growing media in a nutrient free substrate.

Only a nutrient free basal substrate allows to evaluate, for each growing media amendment, the capability i) to buffer the substrate pH decrease following to the addition of acid nutrients solution; ii) to maintain low biological activity thus avoiding a fast substrate degradation and losses of good physical-chemical properties; iii) to increase the N efficiency by avoiding N losses in the environment and resulting in a reduced needs of N fertilizer. All the above-mentioned properties are highly required for the successful use of a growing media (Raviv and Lieth, 2008). Moreover, it is of high importance to evaluate pure BC in a basal substrate, to understand if BC alone could be an alternative to peat or others peat based growing media.

Low N use efficiency (NUE) in high N input nursery crops is a concern for both the economical and environmental sustainability of the system. Therefore, finding new growing media able to maximize the NUE is of crucial importance. To this end, BC has several agronomical properties that makes it particularly attractive as component for growing media. BC is characterized for its highly recalcitrant carbon content (Novak et al., 2010), is a lightweight compound and has a high WHC due to its small pores (e.g. Kammann et al., 2011; Novak et al., 2009). BC demonstrated to enhance nutrients retention in highly weathered soils (Lehmann et al., 2003), particularly reducing NH_4^+ and nitrate (NO_3^-) leaching (Mizuta et al., 2004; Taghizadeh-Toosi et al.,2012; Yao et al., 2012). The higher WHC together with the improved nutrients retention have been suggested as the main causes of the increase in water productivity (WP) and nutrient use efficiency observed in weathered soil amended with BC. Enhanced crop growth is often a consequence of BC application due to the larger amount of available nutrients carried on its surface (Steiner et al., 2008). On the other hand the BC capability to retain nutrients could result in an immobilisation of nutrients, thus in a reduced nutrient availability, which would increase agricultural costs (Novak et al., 2010). To our knowledge only few studies (e.g. Magrini-Bair et al., 2009; Radlein et al., 1997; Taghizadeh-Toosi et al, 2012) test the effect of N enriched biochar by adding nutrients to BC prior to soil incorporation, trying to clarify if charcoal can act as a carrier for N fertilizer, enhance the N retention and affect the plant N availability, finally influencing the use efficiency of N (Clough et al., 2013).

In this study the suitability of BC as substitute for peat in growing media was compared to other peat substitute: perlite and zeolite. We aim to answer the following questions: 1) is biochar a suitable component of soilless growing media for nursery cultivation in terms of crop production and water use efficiency? 2) In the case of distribution of N fertilizer directly on BC surface, is the capability of BC to retain N affecting the NUE by altering the plant N availability?

To this scopes, *Lolium perenne* (L.) plants were grown in pots with five different mixed substrates with two fertilization levels: not fertilized (no_N_f) and fertilized. The distribution of N fertilizer was performed according with two different methods: by application on the previously mixed substrate (mix_N_f) or by soaking each amendment in a N fertilizer solution (amend_N_f) before mixing it with the base substrate (sand). The quality and effectiveness of each growing media component were evaluated in terms of plant growth performance, mineral nutrients content, NUE, WP and soil enzymatic activity as bioindicator of substrate stability.

3.2 Materials and methods

3.2.1 Greenhouse conditions and experimental set-up

The pot experiment was conducted at the experimental farm of the University of Udine, in Northern Italy (lat. 46°2'15.12"N; long. 13°13'33.24, alt. 60 m) in a polyethylene-covered greenhouse with natural daylight conditions. Air temperature and humidity were continuously monitored and recorded every 30 min with a Vaisala Sensor (HMP45C). Average air temperature during the experiment was 16.0 °C and

21.5 °C, and average air moisture was 58% and 53% during the first and the second growing cycle, respectively. Perennial ryegrass (*Lolium perenne*, L.) was grown in 1.1 L round-base plastic pots (14 cm top-diameter and 12 cm height). Perennial ryegrass was chosen because of its high requirement in terms of water and N-fertilization, the high developmental growth rate, and a good re-growth capability allowing for subsequent growing cycles. Seeds were sown at a rate of 4 mg cm^{-2} (400 kg ha^{-1}). To avoid any moisture stress each pot was weighted and irrigated with tap water twice a week in order to offset evapotranspiration losses. To prevent any possible water leaching, each pot was positioned on a saucer.

3.2.1.1 Soil amendments

The base substrate consisted of pure fine sand (type ‘France 70’, Colombo Bolla S.P.A., Italy), with particle size smaller than 0.21 mm for 95 % by weight, mixed with the different soil amendments in a constant 9:1 (v:v) pure sand: amendment ratio. Pure sand was selected as base substrate for its nutrient-free content and inert behaviour referred to CEC. The substrate amendments compared in this study were: peat, zeolite, perlite and two biochar (BC1 and BC2). All the pots were filled with 900 g of sand and the quantity of amendment was determined to keep the total volume constant in the pots, so that the following quantities were added: 6 g, 60 g, 18 g, 14 g and 16 g dry weight of perlite, zeolite, peat, BC1 and BC2, respectively. The treatment that received no amendment (CN) was represented by 1000 g of pure sand. Peat was a commercial product for horticultural plant production, composed of 50% white and 50% black peat, with no added fertilizer, pH of 5.9 (1:2.5 ratio in KCl 1M). Zeolite, a naturally occurred alumino-silicate mineral with a rigid three dimensional crystal structure with voids and channel, was selected for its large internal surface area which determines high CEC and WHC (e.g. Williams and Nelson, 1997). Zeolite is also known to adsorb nutrients in cationic forms, therefore improving their use efficiency as demonstrated for N (Sepaskhah and Barzegar, 2010; Zwingmann et al., 2009), and K (Gül et al., 2005). The zeolite used in this experiment was a commercial product (Adsorbo, E’quo SRL, Italy) coarse textured, with particle sizes varying between 2.5 and 5 mm, selected because made of 90% of clinoptilolite, characterized by a high affinity and selectivity for NH_4^+ (Hedstrom, 2001). Perlite is a lightweight, chemically inert, pH-neutral, sterile amendment which can last for several years. The perlite was characterized by particle sizes comprised, for the 80% by weight, between 2 mm and 5 mm and the remaining 20 % by weight with a diameter smaller than 2 mm. It has no buffering capacity and contains a reduced amount of mineral nutrients. It is very porous and has a strong capillary action that allows it to hold 3-4 times its weight of water. BC1 and BC2 were both obtained by pyrolysis at temperatures of approximately 500 °C. BC1 derived from fruit trees pruning residues and was produced in a discontinuous pyrolysis plant (Romagna Carbone, Bagnacavallo, RA, Italy). BC2 was a commercial horticultural charcoal (Lakeland Coppice Products, England) obtained from coppiced woodlands (beech, hazel, oak, birch) pyrolysed in a transportable ring kiln (215 cm in diameter, holding approximately two tons of hardwood). The pH of BC1 and BC2 (1:2.5 biochar: KCl 1M ratio) was 9.8 and 9.3, respectively. The elemental ratio were 0.30 and 0.05 (H/C) and 0.12 and 0.01 (O/C) for BC1 and BC2, respectively. BCs were grinded and sieved at 5 mm before using in order to standardize their particles sizes. All the amendments used were characterized for their total C and N content, with a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2, Table 3.1). Nutrient compositions was determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES, Varian, Vista MPX; Table 3.2)

Table 3. 1 Chemical composition (mg·kg^{-1}) of substrate components

Substrate	**C_{org}**	**N_{tot}**	**C:N**	**P_{tot}**	**pH**
Sand	0.1	b.d.l.	--	3.83	6.7
Orchard Pruning BC	578.1	9.1	74	23300	9.8
Coppice wood BC	818.3	3.7	258	481	9.2
Peat	227.6	4.6	58	814	5.9
Perlite	0.2	b.d.l.	--	38	
Zeolite	0.2	b.d.l.	--	78	

Table 3. 2 Nutrients composition of substrate components (mg kg^{-1}).

Substrate(1)	Ca	Cu	Fe	K	Mg	P	Mn	Zn
CN	b.d.l.	b.d.l.	211	26	8	3.83	0.02	b.d.l.
BC1	25000	97	333	13900.	28700	23300	84	104
BC2	12270	b.d.l.	626	5166	1132	481	119	b.d.l.
Zeolite	14481	b.d.l.	6227	18759	2447	78	186	12
Peat	19878	11	1217	1368	1444	814	79.	2
Perlite	1390	3	1726	1555	240	38	41	19

(1) CN: Control (pure sand without amendments); BC1: tree pruning biochar; BC2: coppice wood biochar.

3.2.1.2 N fertilization treatments

N fertilizer was applied with two methods: 1) directly on the amendment surface before mixing it with the pure sand (amend_N_f) or 2) on the substrate mixture (sand plus amendment) after seed germination (mix_ N_f). Moreover, each soil mixture was also used in combination with no N fertilization (no_N_f). The amend_N_f treatment consisted in soaking and shaking each amendment with 550 μl of a solution of NH_4NO_3 (1M), for 15 days at 25 °C in a climatic chamber, before mixing it with the sand. The amount of N used was equivalent to 150 mg pot^{-1} of N. This rate was expected to create sub-optimal N conditions for sward growth (Personeni & Loiseau, 2005) in order to investigate the capability of BC and zeolite to retain N, reducing its availability for plant. Mix_N_f treatment was obtained by distributing the N fertilizer dose in each pot after seed germination (10 days after sowing), with a solution of NH_4NO_3 in 20 mL of Milli-Q water. The no_N_f treatments received only 20 mL Milli-Q water.

3.2.2 Plant measurements

3.2.2.1 Biomass production

Lolium perenne production was evaluated at 31 and at 57 days after sowing (DAS). The first harvest was performed cutting the above-ground biomass of *L. perenne* at 3 cm above the substrate level in order to allow plant re-growth. During the second harvest the above-ground biomass was cut at the soil surface level, seedling were counted and below-ground biomass was collected by carefully washing roots present in each pot with a sieve (mesh size 2 mm) and extracting amendment particles by using lab tweezers. The above- and below-ground biomass were oven-dried at 60°C for 4-5 days until stable weight. Total plant N content was evaluated considering both above- and below-ground contents to allow calculation of NUE at the whole plant level, while the nutrients content such as P, K, Ca, Mg, Fe, Mn, Cu, Zn was evaluated considering only above-ground portion.

3.2.2.2 Plant NUE

Above- and below-ground biomass, collected at the two harvests, were finely ground (< 200 μm) and approximately 5 mg sub-sample was analysed, in triplicate, for total N content, with a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2). The NUE was calculated as the ratio of plant biomass (dry matter) at the two harvest and total N input, taking into account the inputs distributed in each pot with the irrigation water, the N-fertilizer and the amendment used (Table 3.3). The N inputs derived from the water supplied were quantified on water samples collected one time per week during the experimental period, and analysed for the total N content by using a Shimadzu TOC-V analyzer (Shimadzu Corp., Kyoto, Japan) combined with a TNM-1 module for N determination.

Table 3. 3 Weight ratio (% w/w) of mixed substrates and total quantity of growing media (g·pot^{-1}). Total N(N_{gm}) and P (P_{gm}) content of growing media and mineral N added with fertilizer (N_f) and with irrigation water (N_w)**. All results are in mg pot^{-1}.**

	------------------------- **Growing Media** ------------------					----------- **Mineral N Input** ------		
Sub-strate$^{(1)}$	**Sand**	**Amend-ment**	**Total**	**P_{gm}** $^{(2)}$	**N_{gm}**$^{(2)\,(3)}$	**N_f**$^{(4)}$	**N_w** $^{(5)}$	**Total Nmin**$^{(6)}$
	(% w/w)		(g·pot^{-1})	------- (mg·pot^{-1}) -		-	(mg·pot^{-1}) ----	
CN	100	-	1000	4	0	150	7-0-7	157-157
BC1	98.4	1.6	914	339	0.13	150	7-10-11	157-160-161
BC2	98.3	1.7	916	11	0.06	150	8-8-8	158-158-158
PT	98.0	2.0	918	18	0.08	150	7-7-7	157-157-157
PR	99.3	0.7	906	4	0	150	8-9-9	158-159-159
ZL	93.7	6.3	960	8	0	150	7-8-8	157-158-158

The growing media used were constituted of pure sand (CN) or of pure sand mixed with different soil amendments, in a constant 9:1 (v:v) pure sand: amendment ratio.
(1) CN: Control growing media without amendments; BC1: tree pruning biochar; BC2: coppice wood biochar PT : peat ; PR : perlite ; ZL : zeolite.
(2) Total P content and total N content of growing media
(3) it refers also to No_N_f (no N-fertilizer) treatments.
(4) N mineral fertilizer added in amend_N_f (N-fertilizer soaked in suspension with the amendment) and mix_ N_f (N-fertilizer solution applied to growing media) .
(5) N added with irrigation water in No_N_f - amend_N_f - mix_ N_f treatments.
(6) Total mineral N (fertilizer +amendment + water) added in No_N_f - amend_N_f - mix_ N_f treatments

3.2.2.3 Water consumption and water productivity.

The water consumption was estimated once a week, as the difference between two consecutive pot weights. After seedlings germination all pots were provided with water until reaching the maximum water holding capacity. The water productivity (WP) was calculated as the ratio of total dry biomass produced (above- and below-ground) per water consumption per each pot, in the entire experimental period (e.g., Sepaskhah and Barzegar, 2010; Kammann et al.2011).

3.2.2.4 Substrate total soluble C and N, available P.

At the end of the experiment, the substrates were evaluated in terms of pH, total soluble C, total soluble N and P availability. Total C and N content were evaluated on a 5 mg air-dried substrate per pot, in triplicate, with a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2). Plant available inorganic P was estimated on 1.5 mg of soil, in duplicate (Olsen et al.,1954). Soluble C and N were evaluated on 5 g of substrate (dry weight basis) extracted with 20 mL of 0.5 potassium sulphate for 30 minutes. After centrifugation (5 minutes at 1000 g), supernatant was filtered using 0.7 µm glass fibre filter (Whatman GF-F). Soluble C and N were determined with a Shimadzu TOC-V analyzer (Shimadzu Corp., Kyoto, Japan) combined with a TNM-1 module for N determination.

3.2.2.5 Substrate Enzymatic Activities

Soil Enzymatic Activity (EA) provides information on the metabolic status of the microbial population and on physico-chemical conditions of the soil. The following EA were tested: acid- and alkaline-phosphatase (respectively acP, alkP) and leucine-aminopeptidase (Lap). Substrates EA were determined in substrate extracts obtained upon applying an extraction/desorption procedure (Fornasier and Margon, 2007). Extracts were obtained using 500 mg of soil and 1.2 mL of extractant (3% lysozyme) in 2-mL Eppendorf tubes containing 0.4 mL of 1 mm diameter ceramic beads and 0.4 mL of 100 micron glass beads. Tubes were shaken for 3 minutes at 30 strokes s-1 using a Retsch 400 beating mill then centrifuged at 15,000 *g* for 3 minutes. Aliquots of supernatants were dispensed in 384-well microplates with appropriate buffer to determine EA using fluorescent 4-Methyl-umbelliferyl- and 4-aminomethyl-coumarine-based substrates. Results were expressed as nano-moles of 4-methylumbelliferone or 4-aminomethylcoumarine per g of dry soil.

3.2.2.6 Statistical analyses

The experiment was arranged as a completely randomized block design with 17 treatments, each of them replicated five times (a total of 85 pots). Significant differences among the treatments were evaluated with two-ways analysis of variance (ANOVA), with two main factors, substrate amendment and N-fertilizer treatment, using the software CoHort, version 6.204. For the N-fertilizer treatment, the sand without addition of any soil amendment was used as a control with the comparisons only among the Mix_N_f treatment: it was not possible to include an amend_N_f treatment since no amendment was added in the pure sand.

Plant N availability, concentration, biomass content and NUE were analyzed considering in the ANOVA only the soil amendments and N distribution methods (Amend Nf vs. Mix Nf without the sand treatment). When main factors or interactions were significant, treatment means separation was performed with the Tukey test (P<0.05).

3.3 Results

3.3.1 Above- and below-ground biomass production

The seedlings survivorship was not influenced by the treatments, thus indicating the absence of an effect of number of plants on the total biomass. The total dry biomass (DW_{tot}), expressed as sum of the above-ground biomass collected in the two harvests and below-ground biomass, was affected by both the N-fertilization treatment and the substrate used (N-fert × amendment $P<0.05$; Fig. 3.1; Table 3.4). The N fertilization treatment did not affect perlite and sand. These last two amendments, when not fertilized, had a significantly lower DW_{tot} compared to all the other combinations of factors. Within the No_N_f treatment peat, BC1, BC2 and zeolite did not differed among them, while all were positively (and significantly) influenced by the addition of N fertilizer. No differences derived from the N distribution methods in case of BC2 and zeolite. Conversely, in the case of BC1, Mix_N_f was significantly higher compared to $Amend\_N_f$ and to all the other combinations of factors. Peat $Amend\_N_f$ was significantly higher than peat Mix_Nf and significantly lower than BC1 $Amend\_N_f$.

The above-ground biomass at the first harvest (Ab1) was less productive than the second one (Fig. 3.1; Table 3.4). In both harvests, fertilized BC1 was the most productive treatment but, while at the first harvest BC1 $Amend\_N_f$ produced significantly more than BC1 Mix_N_f (+19%), in the second harvest this relation was reversed (+30% in BC1 Mix_N_f). In order to give an idea of the root:shoot ratio at the end of the experiment, only the final harvest will be described. Above- and below-ground biomass production at the second harvest (Ab2 and Bl, respectively) were significantly affected by both the N-fertilization and the substrate used (N-fertilizer × amendment $P<0.001$). BC1 mix_N_f had the highest Ab2 and Bl compared to all the other treatments even though its Bl was not significantly different from BC1 $amend\_N_f$ and peat $amend\_N_f$. Zeolite, sand and perlite were never influenced by the N fertilization treatment. Conversely, the addition of the N fertilizer significantly increased Ab2 in case of both BCs and of peat and Bl in case of BC1 and peat. BC1 mix_N_f had the highest increase compared to its respective not fertilized (+ 342 % and +132 % of Ab2 and Bl, respectively). BC1 $amend\_N_f$ compared to BC1 No_N_f significantly increased both its Ab2 (+240 %) and Bl, (+114 %). Also peat significantly increased its Ab2 when fertilized with N: both in amend_N_f (99 %) and in mix_N_f (+ 80 %). In case of BC2 Ab2 increased of +102 % (amend_N_f) and of +68 % (mix_N_f) compared to the respective amendment not fertilized, while its Bl was not influenced by the N treatment. In case of N provision, BC1 mix_N_f was the amendment that most stimulated the biomass increase compared to sand (+ 406 % and + 244 % of Ab2 and Bl, respectively). When the N fertilizer was provided, no significant differences derived from the method of application of it, in terms of both Ab2 and Bl, but two exception were observed: the Ab2 of BC1 was significantly higher in Mix_N_f than in Amend_N_f, and, conversely, the Bl of peat was significantly increased in Amend_N_f than in Mix_N_f (+31%).

Within the No_N_f treatment no differences among amendments were observed in terms of Ab2, while in terms of Bl peat caused a significant increase compared to sand and perlite. This latter treatment had a significantly lower Bl compared to all the other not fertilized substrates.

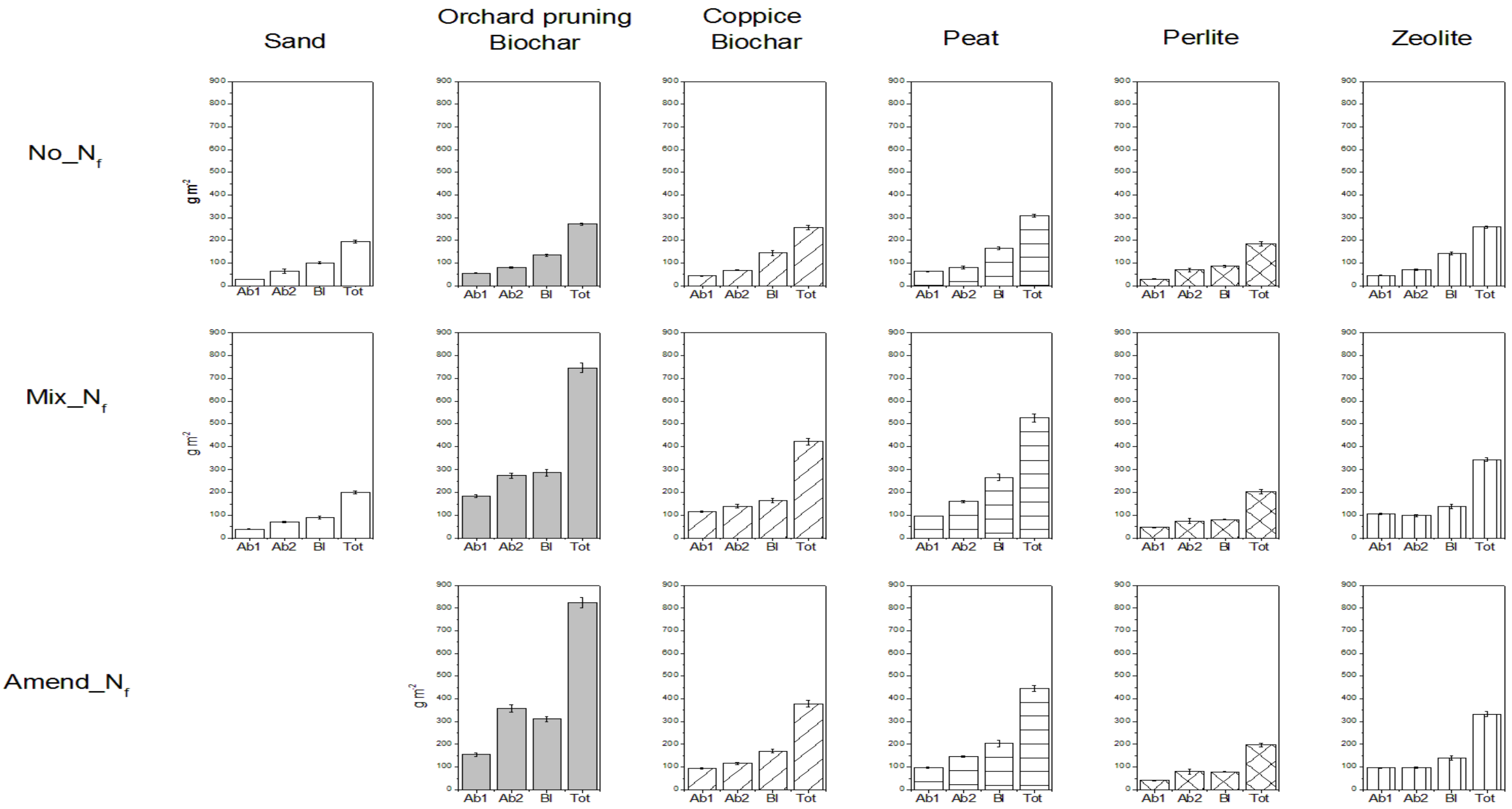

Figure 3. 1 Biomass production (Dry weight, g m^{-2}). Above-ground biomass produced at 1st harvest (Ab1) and at the 2nd harvest (Ab2); below ground biomass collected at the experimental end (Bl) and Total biomass (Tot) as sum of above-ground biomass collected in 2 harvests and below-ground biomass collected at the end of the experiment. Plants of Lolium perenne (James N. Galloway) were grown with different soil amendments and with three N-fertilizer treatments: 1) no N fertiliser (No_N_f); 2) N-fertiliser applied directly on the amendment surface before mixing (amend_N_f) or 3) N-fertiliser applied on the substrate mixture (mix_ N_f). Soil amendments compared were: peat, perlite, zeolite, orchard pruning biochar (BC1) and coppice wood biochar (BC2). The control was constituted of sand. Means ± SE (n = 5).

Table 3. 4 Analysis of Variance and mean values for Above ground Biomass (g m^{-2}) of the 1st harvest (Ab1) and 2nd harvest (Ab2), Below ground biomass (Bl, g m^{-2}) at 2nd harvest, Total Biomass (Tot, g m^{-2}) (Tot= Ab1+Ab2+Bl) and Root:Shoot ratio (R:S).

Factor		**Ab1**	**Ab2**	**Bl**	**TOT**	**R:S**
S (Substrate)		*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001
N_f (N fertilizer)		*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001
S x N_f		*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001	ns
N_Fertilizer(2)	**Substrate(1)**			(g·m^{-2})		
No_N_f	**CN**	28.27	64.61	101.14	194.02	1.69
	BC1	56.14	80.92	134.17	271.23	1.66
	BC2	44.30	68.96	144.16	257.42	2.11
	Peat	62.70	80.73	164.70	308.13	2.08
	Perlite	29.41	69.46	85.48	184.35	1.28
	Zeolite	45.30	71.14	142.25	258.69	2.03
Mix_N_f	**CN**	36.67	70.70	90.46	197.83	1.30
	BC1	154.26	357.66	310.97	822.89	0.87
	BC2	93.76	115.71	169.67	379.14	1.47
	Peat	97.25	145.34	203.19	445.78	1.40
	Perlite	40.23	79.50	78.31	198.04	1.08
	Zeolite	95.79	97.51	139.53	332.83	1.44
Amend_N_f	**BC1**	183.90	274.84	287.62	746.36	1.04
	BC2	116.47	139.33	164.71	420.51	1.20
	Peat	97.64	160.68	267.02	525.34	1.66
	Perlite	46.73	75.50	81.31	203.54	1.17
	Zeolite	105.89	98.54	139.72	344.15	1.43
LSD 0.05		8.45	21.32	26.77	33.80	0.41

(1) CN: Control growing media without amendments (pure sand); BC1:orchard pruning biochar; BC2: coppice wood biochar.

(2 No_N_f: no N-fertilizer; amend_N_f: N-fertilizer soaked in suspension with the amendment; mix_ N_f: N-fertilizer solution applied to growing media.

3.3.2 Nitrogen and water availability

3.3.2.1 Nitrogen concentration in above- and below-ground biomass

A significant interaction ($P<0.001$) between the N-fertilization method (Amend_N_f vs. Mix_N_f) and the amendment affected the N concentration in the above-ground biomass at the first harvest, while only the amendment treatment affected, at the second harvest, the N concentration in both above- and below-ground biomass (Table 3.5). At the first harvest Mix_N_f was significantly higher than Amend_N_f, in case of BC1 and BC2 (+44% and +28%, respectively), nevertheless this effect did not persist at the second harvest, both for above- and below-ground biomass. At the first harvest, BC1 amend_N_f and BC2 amend_N_f resulted in the lowest N concentration in the above-ground biomass compared to all the other fertilized substrates, although BC2 amend_N_f, was not significantly lower than zeolite. BC1, resulted in a significantly lower N concentration, also at the second harvest, both in terms of above and below-ground biomass, compared to the other substrates. The N concentration in the above-ground biomass was the highest in peat and perlite at the first harvest, in perlite and zeolite at the second harvest compared to the rest of substrates. Referring to the below-ground biomass, the N concentration was the highest in perlite, although perlite Mix_N_f was not significantly higher than zeolite and BC2 Mix_N_f.

Table 3. 5 Analysis of Variance and mean values for N concentration (mg kg^{-1}) in above ground dry matter collected at first (Ab1) and second (Ab2) harvest and in below ground biomass (Bl)

Factor				
S (Substrate)		*** P<0.001	*** P<0.001	*** P<0.001
N_f (N fertile)		*** P<0.001	ns	ns
S x N_f		*** P<0.001	ns	ns
N_Fertilizer (2)	**Substrate**(1)	mg kg^{-1}		
		Ab_1	Ab_2	Bl
Mix_N_f	**BC1**	40.31	11.29	7.14
	BC2	41.02	37.59	16.89
	Peat	60.05	34.20	14.20
	Perlite	58.74	48.92	21.62
	Zeolite	35.14	43.58	18.93
Amend_N_f	**BC1**	28.04	9.07	6.96
	BC2	32.12	32.44	15.57
	Peat	56.61	32.46	12.26
	Perlite	58.21	50.43	23.95
	Zeolite	34.25	45.32	18.37
LSD 0.05		2.56	4.40	2.97

(1)BC1:orchard pruning biochar; BC2: coppice wood Biochar.
(2)Amend_N_f: N-fertiliser soaked in suspension with the amendment; mix_ N_f: N-fertiliser solution applied to growing media.

3.3.2.2 Total N content in above- and below-ground biomass

The total N content in the above- and below-ground biomass collected in the two harvests was significantly affected by the interaction between the N-fertilization method and the amendment used ($P<0.001$;Table 3.6). The first considered factor affected significantly the total N content only in case of BC1, where mix_N_f resulted higher than amend_N_f (+29 %), while no differences where observed in case of the other substrates. The highest total N content resulted in peat (mix_N_f and amend_N_f) and in BC1 mix_N_f. Intermediate total N content was observed with fertilized BC2 and zeolite and for BC1 amend_N_f, while the lowest content was observed with perlite.

Table 3. 6 Analysis of Variance and mean values for N uptake in: Above ground Biomass of the 1st plus 2nd harvest (N_{UAb}), below ground biomass (N_{UBl}) total biomass (N_{UTOT}). P uptake in above ground biomass of the 1st plus 2nd harvest (P_{UAb}).

Factor		N_{UAb}	N_{UBl}	N_{UTOT}	P_{UAb}
S (Substrate)		*** $P<0.001$	*** $P<0.001$	*** $P<0.001$	*** $P<0.001$
N_f (N fertile)		* $P<0.05$	ns	ns	ns
S x N_f		*** $P<0.001$	ns	*** $P<0.001$	** $P<0.016$
N_Fertilizer [(2)]	**Substrate[(1)]**		($g \cdot m^{-2}$)		
Mix_N_f	**BC1**	10.26	2.22	12.48	0.66
	BC2	8.16	2.86	11.03	0.12
	Peat	10.78	2.79	13.97	0.25
	Perlite	6.22	1.70	7.92	0.12
	Zeolite	7.62	2.63	10.25	0.11
Amend_N_f	**BC1**	7.64	2.00	9.64	0.50
	BC2	8.28	2.54	10.82	0.16
	Peat	10.74	3.23	13.58	0.26
	Perlite	6.46	1.95	8.41	0.14
	Zeolite	8.07	2.57	10.65	0.11
LSD 0.05		0.83	0.59	0.96	0.09

(1)BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2)Amend_N_f : N-fertilizer soaked in suspension with the amendment; mix_ N_f : N-fertilizer solution applied to growing media.

3.3.2.3 Total soluble N in the substrate

The total soluble N in the substrate at experimental end was not influenced by the interaction between the method of N fertilizer application and the amendment treatment. Only the amendment used affected the total soluble N measured at the end of the experiment (Table 3.7). Perlite, followed by zeolite Amend_N_f, resulted significantly higher compared to both BCs and peat. On their part, peat and BC2 Mix_N_f resulted in a not significantly lower amount of total available N at the end of the experiment compared to zeolite x amend_N_f.

Table 3. 7 Analysis of variance and mean values for Soluble N (N_{SOL}) in growing media at the end of the experiment.

Factor		N_{SOL}
S (Substrate)		*** P<0.001
N_f (N fertile)		ns
S x N_f		ns
N_Fertilizer (2)	**Substrate**(1)	mg kg^{-1}
Mix_N_f	**BC1**	0.10
	BC2	1.12
	Peat	0.51
	Perlite	5.15
	Zeolite	2.27
Amend_N_f	**BC1**	0.22
	BC2	0.21
	Peat	0.43
	Perlite	5.15
	Zeolite	3.78
LSD 0.05		1.19

(1)BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2)amend_N_f : N-fertilizer soaked in suspension with the amendment; mix_ N_f : N-fertilizer solution applied to growing media.

3.3.2.4 Plant NUE

NUE was statistically affected by the interaction between the amendment treatment and the method of N fertilizer application ($P<0.001$), resulting, in case of peat, higher in Amend_N_f compared to Mix_N_f. The method of N fertilizer distribution did not cause any significant effect in case of the other substrates (Fig.3.2). BC1 resulted the most efficient substrate in the use of total N inputs, significantly compared to all the other considered substrates. On their part zeolite, peat Amend_N_f and BC2 Amend_N_f were equally efficient in using the N inputs, while perlite resulted in the lowest NUE compared to all the other substrates.

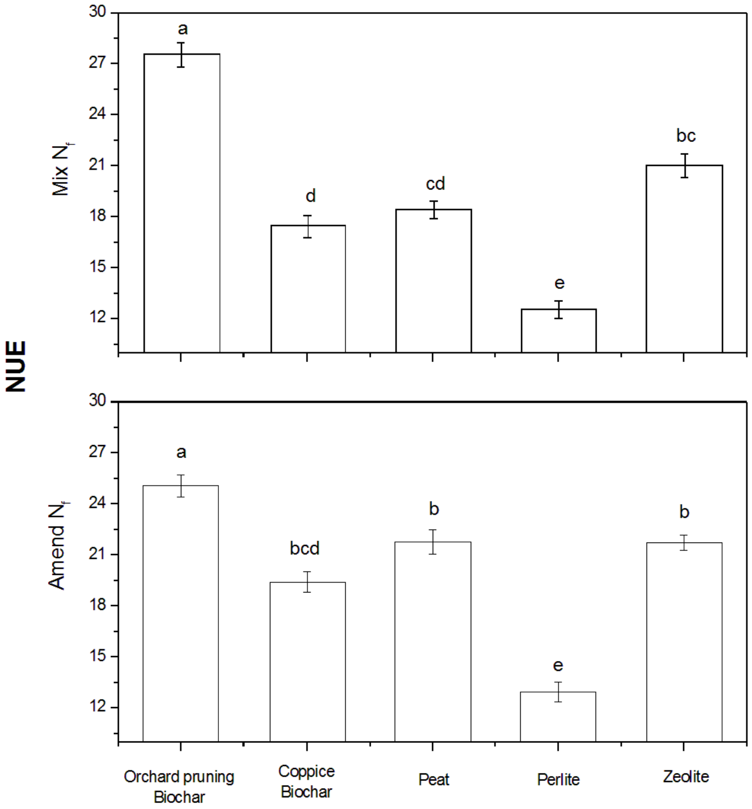

Figure 3. 2 NUE as ratio between the total biomass (g m^{-2}), as sum of above- and below-ground biomass collected in two harvests, and the total N inputs (g m^{-2}), as sum of N distributed with irrigation, with the substrate amendment and with the N fertilization treatment. Only the 2 N treatments where N fertilizer was distributed are represented: 1) N-fertiliser applied directly on the amendment surface before mixing (amend_N_f) or 2) N-fertiliser applied on the substrate mixture (mix_ N_f). Soil amendments compared were: peat, zeolite, perlite, and two biochar (BC1 and BC2). Means ± SE (n = 5). Different letters indicate treatment means significantly different at $P < 0.05$

3.3.2.5 Water consumption and water productivity

Both water consumption (W_c) and water productivity (WP) were statistically affected by the interaction between the two considered factors ($P<0.0001$; data not shown). The absence of supplied N determined a significant reduced W_c and WP while the method of N distribution never affected these two parameters. Within the not fertilized treatment, the WP was significantly higher in peat and BC1 compared to sand and perlite which resulted both in the lowest WP. The N addition positively affected both WP and Wc of all the substrates with the exception of perlite and sand. W_c and WP resulted both significantly higher in case of fertilized BC1 compared to all the other combinations of factors. BC1 mix_N_f increase of 42 % and of 113 % compared to BC1 no_N_f, in terms of W_c, and WP, respectively. The second important increment was observed in peat Amend_N_f which increase of 10 % and 48 % compared to peat No_N_f in terms of W_c and WP, respectively.

3.3.3 Substrate P availability

P availability measured at the end of the experiment was significantly affected by the interactions between the two studied factors ($P<0.001$, Table 3.8), particularly resulting, in BC1, significantly higher in No_N_f compared to both Amend_N_f and Mix_N_f. BC1 had a significantly higher amount of available P compared to the rest of the combination treatments. The application method of N fertiliser (Amend_Nf vs. Mix_Nf) did not affect this parameter.

Table 3. 8 Analysis of variance and mean values for available P (P_{Olsen}) in growing media at the end of the experiment (mg kg^{-1}).

Factor		P_{Olsen} (mg kg^{-1})
S (Substrate)		*** $P<0.001$
N_f (N fertile)		*** $P<0.001$
S x N_f		*** $P<0.001$
N-Fertilizer $^{(2)}$	**Substrate**$^{(1)}$	
No_N_f	**CN**	0.14
	BC1	6.74
	BC2	0.42
	Peat	0.47
	Perlite	0.21
	Zeolite	0.25
Mix_N_f	**CN**	0.08
	BC1	1.73
	BC2	0.26
	Peat	0.24
	Perlite	0.12
	Zeolite	0.08
Amend_N_f	**BC1**	1.88
	BC2	0.21
	Peat	0.28
	Perlite	0.40
	Zeolite	0.02
LSD 0.05		0.492

(1) CN: Control growing media without amendments; BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2) No_N_f : no N-fertilizer; amend_N_f : N-fertilizer soaked in suspension with the amendment; mix_ N_f: N-fertilizer solution applied to growing media.

3.3.4 Nutrients content in above-ground biomass

Both the N-treatment and the amendment used affected P, K, Ca, Mn, Cu and Cd total content in the above-ground biomass collected in the two harvest (N-fertilizer × amendment $P<0.001$). The total P content of the biomass produced from BC1 was significantly higher compared to all the rest of the amendments, and was also significantly affected by the N application method, resulting higher in the Mix_N_f compared to the Amend_N_f and in the latter compared to No_N_f (Table 3.9). No differences were observed among BC1 No_N_f and peat, which was, on its part, not affected by the N fertilization treatment.

K content in the total above ground biomass was significantly higher in fertilized BC1 compared to the rest of the treatments. Fertilized BC2 and zeolite resulted not different among each other, but both were significantly higher compared to perlite, sand and peat, but not than peat Amend_N_f.

The Ca total biomass content was significantly higher in fertilized peat compared to all the other combinations of factors. Also within the No_N_f treatment, peat had a significantly higher Ca content compared to both BCs, but not compared to the other substrates.

The Cu leaf content was significantly higher in fertilized BC1 compared to all the other combinations of factors, but in case of Amend_N_f treatment BC1 was not significantly different than BC2 and peat. Also Mn content was affected by the N treatment and the amendment applied.

Table 3. 9 Nutrient uptake with the total above-ground biomass collected at the two harvest (mg m^{-2}). Different letters indicate statistically different values ($P<0.05$)

N-Fertilizer(2)	**Substrate(1)**	**P**		**K**		**Ca**		**Mn**		**Cu**	
No_N_f	**CN**	109	g	335	f	901	cdef	1.14	h	0.44	c
	BC1	357	c	4187	bcde	548	f	4.62	gh	0.91	c
	BC2	108	g	2106	cdef	578	ef	4.28	gh	0.60	c
	Peat	247	cdef	743	f	1358	bcd	13.15	cde	0.74	c
	Perlite	103	g	339	f	835	def	1.22	h	0.68	c
	Zeolite	110	g	2636	cdef	878	def	4.16	gh	0.76	c
Mix_N_f	**CN**	124	efg	480	f	1513	bcd	3.98	gh	1.05	c
	BC1	662	a	13416	a	1930	b	11.58	def	4.48	a
	BC2	124	efg	4451	bcd	1408	bcd	16.63	gh	0.90	c
	Peat	252	cde	1238	ef	2995	a	20.58	b	1.11	c
	Perlite	125	efg	432	f	1670	bc	4.27	gh	1.13	c
	Zeolite	115	fg	5066	bc	1430	bcd	10.06	defg	1.35	c
Amend_N_f	**BC1**	497	b	11641	a	1335	bcde	7.50	efgh	3.38	ab
	BC2	163	defg	5799	b	1655	bc	29.95	a	2.25	bc
	Peat	258	cd	1321	def	3334	a	29.17	a	1.56	bc
	Perlite	140	defg	528	f	1608	bcd	6.29	fgh	1.37	c
	Zeolite	113	g	4978	bc	1425	bcd	18.41	bc	1.09	c

(1)CN: Control growing media without amendments; BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2)No_N_f : no N-fertilizer; amend_N_f : N-fertilizer soaked in suspension with the amendment; mix_ N_f: N-fertilizer solution applied to growing media.

3.3.5 Stability of the growing media: pH, enzymatic activities, soluble C

3.3.5.1 Substrate pH at the end of the experimental period

The pH of the substrates measured at the end of the experiment was significantly affected by the interaction between the two studied factors (P<0.05, Fig. 3.3). The addition of N caused a significant reduction of pH in substrate with perlite, sand and also in BC1 Mix_N_f, while no changes of pH were observed in peat, zeolite and BC2. The fertilized sand resulted in the lowest pH mean value (8.2), although not significant different from fertilized perlite and zeolite, from peat both No_N_f and Mix_N_f. BC1 No_N_f resulted in the highest pH value (9.9), although not significantly different than BC1 Amend_N_f. On its part, the pH of this last combination of factors, was not significantly higher than in not fertilized perlite and sand, in BC1 Mix_N_f and in BC2, independently from the N-treatment applied to it.

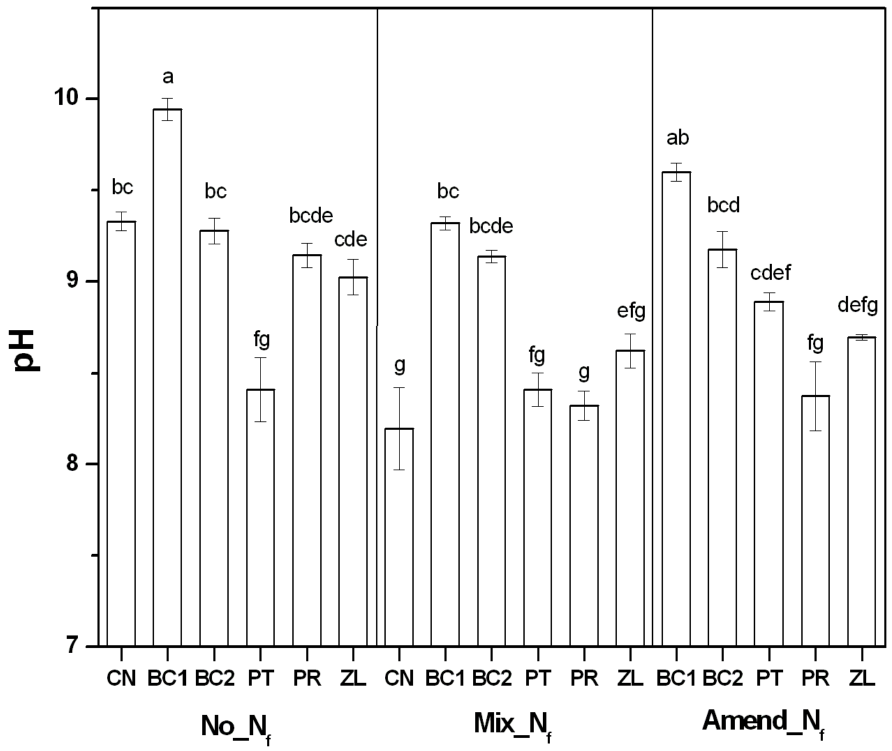

Figure 3. 3 pH of substrates measured at the experimental end. Plants of *Lolium perenne* were grown with different soil amendments and with three N-fertilizer treatments: 1) no N fertilizer (No_N_f); 2) N-fertilizer applied directly on the amendment surface before mixing (amend_N_f) or 3) N-fertilizer applied on the substrate mixture (mix_ N_f). Soil amendments compared were: peat (PT), zeolite (ZL), perlite (PR), and two biochar: orchard pruning biochar (BC1) and coppice wood biochar (BC2). CN represents the base substrate (sand alone). Means ± SE (n = 5). Different letters indicate treatment means significantly different at $P < 0.05$

3.3.5.2 Enzymatic Activity and total soluble carbon

AcP, alkP and Lap activities were affected by the interaction between the two considered factors ($P < 0.001$) and resulted highest in case of peat amend_N_f (Fig.3.4) followed by peat mix_N_f. AcP, alkP and Lap activities were not affected by the N fertilization treatment with the exception of peat, in which the addition of N fertilizer stimulated all the measured EAs, for acP and Lap significantly only in case of amend_N_f (+ 38 % and +197 %, respectively), while for alkP significantly both in case of Amend_N_f and Mix_N_f (+ 219 % and + 110 %, respectively) compared to peat No_N_f. Peat amend_N_f resulted in the higher EAs significantly compared to all the combinations of factors exception for peat mix_N_f, in case of acP and Lap. All the combined treatments other than the fertilized peat were characterized by a not statistically different Lap activity, exception made for BC1 Mix_N_f which resulted significantly higher than fertilized perlite and pure sand (Fig.3.5, Panel C). The alkP in fertilized BC2, zeolite amend_N_f and peat no_N_f, had intermediate values, significantly reduced compared to fertilized peat and significantly increased than in fertilized perlite and pure sand (Fig 3.5, Panel B). On the other hand acP activity in peat Mix_N_f, was significantly higher only compared to perlite, not fertilized biochars and zeolite. Both not fertilized BCs resulted in the lowest acP activity. Such difference was statistically significant only when compared to peat, fertilized sand and BC2 Mix_N_f (Fig 3.5, Panel B).

Total soluble C resulted influenced by the amendment application but not by the N treatment ($P<0.001$; data not shown). Peat Amend_N_f resulted significantly higher when compared to fertilized sand and perlite, to BC2 No_N_f and to zeolite, independently from the N treatment applied ($P<0.05$). Nevertheless total soluble C in peat did not resulted significantly higher than BC1 or in fertilized BC2.

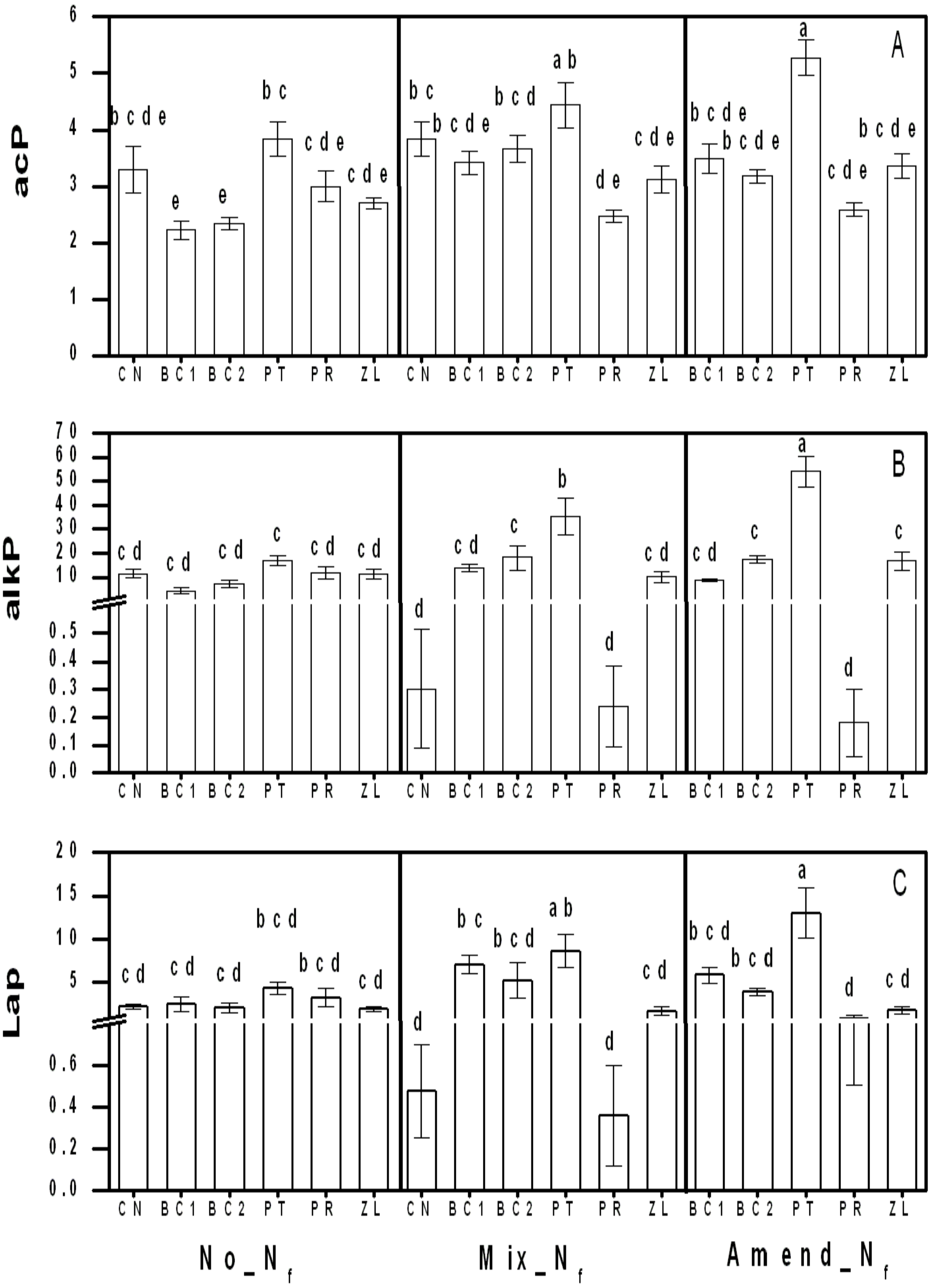

Figure 3.4 **A**. Acid-phosphatase activity; **B**. Alkaline-phosphatase activity; **C**. Leucine-aminopeptidase activity; Plants of *L. perenne* were grown with different soil amendments and with three N-fertilizer treatments: 1) no N fertilizer (No_N_f); 2) N-fertilizer applied directly on the amendment surface before mixing (amend_N_f) or 3) N-fertilizer applied on the substrate mixture (mix_ N_f). Soil amendments compared were: peat (PT), zeolite (ZL), perlite (PR), and two biochar: orchard pruning biochar (BC1) and coppice wood biochar (BC2). CN represents the base substrate(pure sand). Results were expressed as nano-moles of 4-methylumbelliferone or 4-aminomethylcoumarine per g of dry soil. Means ± SE (n = 5). For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

3.4 Discussion

3.4.1 Is biochar a suitable component of soilless growing media for nursery cultivation in terms of biomass production and water use efficiency?

Although the positive effect of BC is largely investigated as soil amendment, only few studies, to date, evaluate the use of BC as peat substitute in growing media. In our study, both BCs were as good as peat or, when provided with N fertilizer, even more effective than peat (BC1) in sustaining biomass production, improving N use efficiency and water productivity. Our results are in line with the outcomes of the meta-analysis conducted by Jeffery et al. (2011) where significant increases in crop productivity were observed when BC was applied with inorganic fertilizer. Our findings are also in agreement to the ones obtained by Kammann et al. (2011), which assessed the effects of BC addition to a sandy soil on *Chenopodium quinoa*, in presence of a complete nutrient solution. *C. quinoa* had a significantly higher total biomass (+ 60 %) WUE and NUE in presence of biochar. Moreover Kammann et al. (2011) found, similarly to this study, that the leaf N concentration was reduced in presence of BC, as consequence of the large increase in plant above-ground biomass (see Section 3.3.2.1). Only few studies to date compared BC with peat as soilless amendment and one of these found greater total biomass in the ornamental *Calathea rotundifolia* grown in a 50% (v:v) biochar:peat substrate as compared to a 100% peat substrate (Tian et al., 2012). The increase obtained after the addition of N, expressed as average between Amend_N_f and Mix_N_f treatment compared to the No_N_f treatment, was highest, in absolute terms, in case of BC1 (+291 %), followed by peat (+89 %) and BC2 (+85 %). This data confirmed the 'synergism' between BC and N fertilizer hypothesized by Steiner et al. 2007.

In spite of the presence of considerable amounts of macronutrients without the addition of N fertilizer BC was not able to improve total biomass production compared to the other amendments. As expected, not much of the applied biochar-N become available for plants via mineralization, neither in case of BC1, despite its lower C/N ratio (74) compared to BC2 (258). This result is consistent with findings by Knicker (2010) according to which the N content in charred cellulose or wood, is highly recalcitrant and stable with respect to mineralization. Gaskin et al. (2010) and Wang et al. (2012) showed that the N present in BC could be either (i) acid hydrolysable extractable N (THN) or (ii) heterocyclic N, the latter not available for plant uptake. The heterocyclic N fraction increases with pyrolysis temperatures or depending on the feedstock (wood or cellulose-BC has higher content compared to grass-BC, Mukherjee & Zimmermann 2013). De la Rosa and Knicker (2011) demonstrated that only 10 % of the N added to the soil with pyrogenic organic matter derived from grass-BC, becomes bio-available. This means that it could be expected that in wood-BC this fraction would be even lower. Further experiments are needed to better clarify the capability of BC to recycle the N contained in the original biomass used for its production.

The significant large increase in total biomass observed in fertilized BC1 compared to all the other amendments, including BC2 (Fig.3.1) could be explained from the plant-available nutrients provided by the BC itself such as P, K, Mg, Mn, Cu and Zn (Table 3.9). These nutrients are demonstrated to be available for plant uptake (e.g. Liu et al., 2012; Novak et al., 2009). BC1 was made from fruit tree pruning, thus from nutrients-rich wood, so many nutrients were recycled yielding a higher productivity compared to all the other amendments considered. In this study leaching was not allowed so it is possible to exclude that the capability of BC to prevent nutrients leaching could have been the mechanism behind the higher biomass observed and also the liming effect of BC observed in acidic soils and indicated as the main cause of P bioavailability (Van Zwieten et al., 2010) would not have played a major role under our experimental conditions as both BCs amended substrate had a pH of approximately 9. On the other hand, total Ca uptake resulted significantly lower in both fertilized BCs compared to the fertilized peat. This result can be explained considering that the release of some nutrients by BC is pH-dependent and, particularly for Ca ions, it is reduced to negligible values when the pH is higher than 8.9 (Silber et al., 2010; Xu et al., 2013) as under our experimental conditions. On the contrary, P and K release is not dependant on the environmental pH conditions (Silber et al., 2010). BC2 did not provide this high availability of mineral nutrients probably because it derived from forest wood, not fertilized as cultivated fruit trees. For this reason we conclude that it is of fundamental importance to consider the origin of the material used for the production of BC for soilless substrate and to favour, at this scope, the use of cultivated crop residues in order to allow the recycle, of mineral nutrients and possibly lower the input of fertilizers in soilless cultivations.

One of the important characteristic of soilless substrates is the capability to maintain alkaline or neutral pH conditions, buffering the acidification derived from the continuous addition of fertilizer solution, which is a widely adopted practice in high intensity soilless production system (Raviv et al., 2002). BC2 showed to be as effective as peat and zeolite in maintaining unchanged the pH value compared to the respective not-fertilized substrate, while BC1 significantly reduced its pH in case of Mix_N_f treatment, maintaining, however, an alkaline pH (9.3).

A further requirement for a good-quality soilless substrate is its biological stability and its resistance to degradation. Previous study (Tian et. al. 2012) demonstrated that while in peat substrate the particle size distribution changes between the start and the end of the experiment, revealing a loss of aggregates, the addition of BC to peat reduced substrate degradation. Additionally BC used as such, was even more stable than 50% mix BC/peat (Tian et al., 2012). In our study total soluble C could be used as an indicator of the media stability as it is positively related to microbial activity (e.g. Zavalloni et al., 2010), together with the alkP and the Lap activities, which are recognized as indicator of microbial respiration and growth (Frankenberger and Dick, 1983; Hernandez and Hobbie, 2010). These parameters resulted higher in peat Amend_N_f, confirming the high biological activity of soilless substrates amended with peat, one of the main known weaknesses of this substrate. The total soluble C content observed in both fertilized BC1 and BC2, which was not significantly lower than in fertilized peat, might be derived from the degradation of the labile C compounds of BCs and is supposed to be reduced over time. Several previous studies demonstrated that BC is a highly stable material, recalcitrant to microbial mineralization either in the short-term period (e.g. 84 days after BC incorporation, Zavalloni et al., 2011 or 25 and 67 days after BC incorporation in Norfolk soil, Novak et al., 2010) and even more in the long-term period (500 days after BC incorporation, Zimmerman et al., 2011). Nevertheless several other studies found that BC had an initial positive priming effect and high decomposition rate, mainly due to the degradation of a limited portion of labile compounds (i.e. volatiles compounds, strongly oxidized parts), which decreased considerably after the first two or three months from its incorporation into the soil (initial rates of 0.05 % day^{-1} and after two to three months 0.0013% day^{-1} in Kuzyakov et al., 2009). Yao et al., 2010 found that the higher release of dissolved organic carbon from BC occurs after 18 h of weathering (0.2%) while after the following 282 h of weathering only an additional amount of 1 % of the initial total organic carbon was released. The effect of BC in stimulating soil microbial activity, was also observed by Castaldi et al.(2011) after three months from BC incorporation into the soil (higher potential soil respiration, net mineralization and denitrification enzyme activities) but it did not persist after 14 months. Several studies hypothesised that the negative priming effect of BC observed in the long-term period is the consequence of BC weathering in the soil, and is possibly due to the switch of BC surface charge from positive to negative, and to functional groups chemistry changes occurring with both biotic and abiotic oxidation (Cheng et al., 2008).

AcP and alkP activities are negatively correlated with substrate P content (Rezende, Assis and Nahas, 2004). This fact probably contributed to the lack of acP and alkP activities observed in BC1 compared to peat. In case of BC2, although P was scarcely available, acP and alkP were significantly lower compared to peat x amend_N_f. We hypothesized that this fact could be related to BC2 high C/N ratio.

3.4.2 In case of enrichment of biochar with N fertilizer, is the capability of BC to retain N affecting the NUE by altering the plant N availability?

The method of N fertilizer distribution did not affect the NUE in case of both BCs used. On the other hand, total N uptake was significantly lower in BC1 Amend_N_f compared to Mix_N_f (- 29%, $P<0.05$). The reduced total N uptake could derive from N gaseous losses, from the formation of ammonia (NH_3) consequent to the contact of NH_4^+-N and BC1 with pH = 9.8, which exceeded the pKa of NH_3/NH_4^+ (9.2 at 25°C). Another possible N loss could have derived from N denitrification on the amendment surface in case of amend_N_f . A further hypothesis is that BC sequestered a portion of N by surface adsorption of NH_4^+-N thus reducing its availability for plant nutrition (Novak et al.,2010; Xu et al., 2013). Nevertheless even if there was an adsorption and a consequent reduction of N availability, this study proved that this reduced N availability was not enough to cause a significant reduction in terms of NUE. BC enriched with N fertilizer is not toxic to plant and is capable to release available N to the soil. Same results were obtained by cultivating maize and bean in a nutrient free artificial soil by Radlein et al. (1997) and unequivocally confirmed in a recent study by the use of ^{15}N (Taghizadeh-Toosi et al., 2012). In the latter study BC was exposed to ^{15}N enriched ammonia (NH_3) and enriched with ^{15}N: it was demonstrated that NH_3 adsorbed onto biochar can provide a source of N for plants when the biochar-NH_3 complex is placed in the soil-plant matrix.

We did not measured the total N content of biochar particles at the end of this experiment but only the total available N in the substrate, which was negligible in BC1, BC2 and peat, independently from the method of N fertilizer application (Section 3.3.2.3). Further studies are required to compare the efficacy of the N enriched BC with the commonly used N fertilizer distribution practices by exactly quantifying, in the first case, the amount of N adsorbed onto BC, released into the soil in plant available forms and subsequently absorbed by plant. Moreover it must be quantified the maximum amount that BC could efficiently retain and vehicle in the soil, acting as a slow release fertilizer.

3.5 Conclusions

BC derived from agricultural nutrient-rich residues (BC1) with N fertilizer, determined higher above- and below-ground biomass productivity, WP and NUE compared to the most used amendments for soilless substrates, included peat. Based on these results, it is possible to conclude that BC has potential as substrate amendment for the composition of soilless media especially when used in not-limited N supply conditions. The enrichment of BC1 with N fertilizer (Amend_N_f) allowed high NUE and WP and did not reduce the amount of total available N in substrates at the end of the experiment, resulting as effective as the application of N fertilizer on the substrate mixture (Mix_N_f). Neither any reduction of available N for plants was observed in case of BC Amend_N_f, nor immobilisation of N occurred in BC2 as it could be expected because of the higher C:N ratio compared to BC1. Moreover we can speculate that in presence of leachate events the NUE here measured in Amend_N_f should increase, as consequence of a better N retention caused by BC negative surface charge and acid functional groups (Steiner et al., 2008; Cheng et al., 2008). Further studies, in presence of leachate events and monitoring the leachate itself are needed to individuate the best practice to enrich BC with N fertilizer before using it as substrate amendment in order to reduce total N losses and possible obtaining a slow N release amendment.

The use of BC from agricultural nutrient-rich residues in soilless substrates might be an innovative and sustainable way to avoid the use of peat from non-renewable peatland areas and favour soilless cultivation. Nevertheless, the present study focused on the BC suitability as amendment of soilless substrate, limiting its evaluation to the BC value in supporting biomass growth and nutrition of *L. perenne*, which is a species with high needs in terms of nitrogen and water supply but is not representative of high marketable values species, (e.g. it is not interesting for its fruits or flowers). Further studies are required to test the effect of BC on the well-being of horticultural and ornamental plants (e.g. species to be sell in pot) or on the quality and/or the time of development of plants' part (flower, fruits, leaves) characterized by high marketable values. The variety of plants cultivated in soilless substrates in pots increased continuously as response to the needs of reducing the consumption of soil. Therefore, it would be a priority to develop a new branch of research aimed at identifying soilless substrate, renewable and environmental friendly, able to satisfy the ever wider range of plants' and market' needs.

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Supplementary material

S.1 Macro and micro-nutrient concentration in above-ground biomass

The concentration of nutrients in the above-ground biomass resulted, in both harvests, highly affected by the interaction between the two studied factors, in case of P, K, Mn and, at the second harvest, also in case of Ca. At the first harvest, Zn leaf concentration was highly influenced by the amendment applied ($P < 0.001$) but this fact was not confirmed at the second harvest. Meanwhile, also the fertilization treatment slightly affected the leaf concentration of Ca, Cu and Fe ($P < 0.05$) with higher concentrations observed in case of N-fertilizer availability.

The leaf P concentration in the above-ground biomass was affected by the interaction among the two studied factor at the first harvest ($P<0.01$) and, even more significantly, at the second harvest ($P<0.0001$). In particular, at the second harvest, BC1 and peat not fertilized, resulted in a significantly higher P leaf concentration compared to the respective fertilized amendment treatment. Not significant differences were observed among BC1 (exception made for BC1 No_N_f), perlite, pure sand and peat, independently from the applied N treatment, and from zeolite No_N_f at the first harvest. Conversely, the fertilized zeolite resulted in a lower value of P concentration compared to BC1 Mix_N_f, BC1 No_N_f , fertilized pure sand and perlite, and to peat No_N_f.

In both the two harvests all the combination containing BC1, BC2 and zeolite resulted in a significantly major leaf concentration of K compared to all the combinations of treatments containing perlite, peat and pure sand. On their part, these last amendments independently from the applied N-treatment, did not result different among them. No differences have been detected between zeolite and BC2 independently from the fertilization treatment applied to them, in both the two harvests. In case of BC1 a positive and significant interaction was observed when the fertilized was mix_ N_f applied, compared to No_N_f treatment but this fact occurred only at the first harvest while at the second harvest no differences derived from the fertilization treatment applied to this amendment. K concentration in the above ground biomass, at the first harvest, was significantly higher in BC1 mix_N_f compared to all the other combination of treatments, exception made for BC1 x amend_N_f. Conversely, at the second harvest, the highest value of K concentration in leaves was observed in case of not fertilized BC1 and was significantly higher compared to all the other combinations of factors, exception made for fertilized zeolite. At the first harvest BC2 No_N_f reported a significantly reduced K concentration in leaves compared to fertilized BC1.

The maximum level of Mn leaf concentration at the first harvest was registered in the combination peat amend_N_f, nevertheless it was not significantly higher than the one observed in case of peat mix_N_f. A significantly positive interaction was observed, in terms of Mn concentration, in case of BC2 and perlite and in case of BC2 and zeolite, respectively at the first and at the second harvest, when the Amend_N_f fertilization was applied, compared to Mix_N_f and to No_N_f treatments. The Mn leaf concentration was not influenced by the N treatment in case of BC1 and CN both at the first and at the second harvest. No differences in Mn concentrations were observed within the No_N_f treatment, independently from the amendment used, exception made for peat that, either at the first either at the second harvest, resulted in a significant higher value of Mn concentration compared to the other not fertilized substrates.For a more detailed description of macro and micro-nutrients leaf concentration in the above ground L. *perenne* biomass please refer to Table S1 and Table S2.

Table S. 1 Nutrients concentration in the above ground biomass at the first harvest (mg kg^{-1})

N-fertilizer[(2)]	Substrate[(1)]	N		P		K		Ca		Mn		Cu	Zn		Fe	Cd
No_N$_f$	**Sand**	14.5	e	1356.6	bcdefg	2246.4	e	7868.7	bcdef	8.5	g	4.8	0.1	b	65.6	0.1
	BC1	9.9	f	2206.8	a	27227.2	bc	2503.7	g	12.8	fg	6.0	0.1	b	31.5	0.0
	BC2	10.4	f	927.6	defg	18152.6	d	3736.2	efg	12.9	fg	3.5	0.0	b	83.3	0.1
	Peat	11.8	ef	1654.2	abcd	3107.6	e	6998.2	bcdefg	36.5	bcde	3.6	0.0	b	60.8	0.1
	Perlite	13.4	ef	1346.2	bcdefg	2627.5	e	7702.7	bcdef	8.0	g	6.1	1.9	b	52.4	0.3
	Zeolite	10.5	f	996.6	cdefg	23815.2	cd	5988.4	cdefg	12.5	fg	7.8	6.0	b	63.0	0.7
Mix_N$_f$	**Sand**	56.1	a	1812.2	abc	3986.2	e	11449.4	ab	25.1	defg	15.8	29.3	ab	98.9	1.1
	BC1	40.5	b	1972.4	ab	41775.2	a	3131.4	fg	20.6	efg	16.7	20.9	b	96.6	0.3
	BC2	40.4	b	720.8	fg	23044.8	cd	4702.7	defg	34.3	cdef	5.8	2.1	ab	58.2	0.0
	Peat	58.8	a	1499.3	abcdef	3718.7	e	8757.2	abcd	58.4	ab	6.3	6.7	b	71.9	0.1
	Perlite	58.1	a	1610.5	abcde	3268.0	e	10112.2	abc	23.5	efg	12.2	24.2	ab	82.0	0.5
	Zeolite	34.9	c	637.4	fg	29562.2	bc	4879.3	defg	18.8	efg	6.9	0.4	b	48.8	0.1
Amend_N$_f$	**BC1**	28.4	d	1323.9	bcdefg	33472.7	ab	2282.9	g	12.4	fg	9.1	0.6	b	248.8	0.1
	BC2	31.8	cd	767.8	efg	27053.6	bc	5077.2	defg	53.3	abc	11.3	11.5	ab	147.0	0.9
	Peat	55.9	a	1316.3	bcdefg	3409.5	e	8583.1	abcd	65.7	a	6.3	8.8	b	73.7	0.1
	Perlite	57.8	a	1920.1	ab	4314.1	e	12914.7	a	48.4	abcd	17.7	54.6	a	132.7	1.8
	Zeolite	34.3	c	544.2	g	26235.1	bcd	4269.7	defg	28.1	defg	5.7	0.0	b	59.1	0.1

(1)CN: Control growing media without amendments; BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2)No_N$_f$: no N-fertilizer; amend_N$_f$: N-fertilizer soaked in suspension with the amendment; mix_ N$_f$: N-fertilizer solution applied to growing media.

Table S. 2 Nutrients concentration in the above ground biomass at the second harvest (mg kg^{-1})

N-fertilizer(2)	Substrate(1)	N		P		K		Ca		Mn		Cu	Zn		Fe	Cd
No_N$_f$	**Sand**	9.7	f	1088.7	c	4230.5	d	10604.5	cd	13.6	j	5.3	78.9	b	102.2	0.8
	BC1	7.5	f	2895.2	a	33035.8	a	5062.6	f	48.9	ghi	7.3	169.5	b	93.0	0.8
	BC2	7.8	f	965.4	cd	18856.3	c	6019.5	ef	53.6	fgh	6.3	58.7	b	59.4	0.6
	Peat	8.7	f	2031.6	b	7278.7	d	12469.3	bc	140.2	bcd	7.0	49.6	b	94.1	0.6
	Perlite	9.2	f	1044.1	c	4113.6	d	9515.2	cd	14.9	ij	7.5	49.1	b	78.2	0.7
	Zeolite	8.6	f	918.8	cde	22384.6	bc	8562.3	de	50.4	gh	5.8	93.4	b	87.5	0.9
Mix_N$_f$	**Sand**	53.3	a	926.3	cde	4953.7	d	16156.7	a	44.4	ghij	7.7	79.4	ab	115.8	0.8
	BC1	11.3	f	1179.3	c	23518.2	bc	4397.8	f	25.3	ghij	6.7	67.8	ab	121.2	0.8
	BC2	37.6	de	604.0	e	23293.4	bc	9138.3	de	121.8	cde	4.0	71.3	b	124.5	0.7
	Peat	34.2	e	914.6	cde	6489.7	d	15820.5	a	109.7	de	4.2	45.4	b	80.0	0.6
	Perlite	48.9	abc	916.7	cde	4162.1	d	16905.3	a	45.3	ghij	9.2	80.9	ab	109.2	0.7
	Zeolite	43.6	cd	676.3	de	28737.1	ab	10833.4	cd	88.1	ef	8.6	74.0	b	124.2	0.9
Amend_N$_f$	**BC1**	9.1	f	1111.1	c	24775.9	bc	3653.6	f	20.7	hij	7.6	61.0	b	44.4	0.6
	BC2	32.4	e	645.3	de	22813.3	bc	8488.5	de	176.1	a	8.5	57.3	ab	114.4	0.7
	Peat	32.5	e	971.4	cd	6630.5	d	16710.9	a	150.5	abc	6.7	64.3	b	97.4	0.6
	Perlite	50.4	ab	904.2	cde	5098.9	d	14796.6	ab	59.6	fg	9.2	109.4	a	143.0	0.7
	Zeolite	45.3	bc	677.2	de	27886.6	ab	10793.4	cd	162.5	ab	6.3	86.5	b	115.8	0.7

(1)CN: Control growing media without amendments; BC1: orchard pruning biochar; BC2: coppice wood biochar.

(2)No_N$_f$: no N-fertilizer; amend_N$_f$: N-fertilizer soaked in suspension with the amendment; mix_ N$_f$: N-fertilizer solution applied to growing media.

CHAPTER 4

General discussion and conclusions

4.1 Introduction

Nitrogen (N) pollution of ground and surface water, N losses into the atmosphere and related reduction in the nitrogen use efficiency (NUE), are major environmental and socio-economic concerns. Biochar interact with the N cycle into the soil and could potentially affect plant N nutrition. This research, with the final aim to clarify the interactions between biochar and the mineral N in the soil, has focused on five major research questions:

1. is it possible to define a maximum adsorption potential of NH_4^+-N onto biochar? Is this potential predictable by assessing the cation exchange capacity (CEC) and the amount of acidic functional groups on biochar surface (section 4.2)?
2. Is the mechanisms of NH_4^+-N adsorption onto biochar dependent on temperature? Does biochar sorption of NH_4^+-N increase by leaching away the minerals present on the biochar surface (section 4.3)?
3. Does the N sorption has negative effects with plant NUE (section 4.4)?
4. Is biochar able to directly provide N to plants when added in a nutrient free substrate of growth (section 4.5)?
5. Is biochar a suitable peat substitute in soilless growing media (section 4.6)?

4.2 NH_4^+-N adsorption onto biochar from aqueous solution

Several studies reported that biochar is able to absorb N-NH_4^+ sometimes even in considerable amounts (Chapter 2, Section 2.1). In the study here reported (Chapter 2, Section 2.3.3) this mechanism was also observed and confirmed. However, contrary to the main current hypothesis, both the CEC and the number of carbon surface functional groups (*ncsf*) could not completely explain the adsorption of NH_4^+-N onto biochar. In fact, by performing batch adsorption experiments, the amount of NH_4^+-N adsorbed largely exceeded the CEC and the *ncsf*. The amount of NH_4^+-N adsorbed onto biochar continued to increase with increasing concentrations of NH_4^+-N in the liquid phase. For this reason the Langmuir adsorption model, which hypothesised the existence of a plateau level in the adsorption of a sorbate onto a sorbent, did not fit experimental data as well as the Freundlich model which, instead, does not expected the existence of a maximum adsorption level. Nevertheless, the Freundlich model is, for its nature, mathematically unable to describe the linear portion of the isotherm curves that were described in Chapter 2. This facts lead to the needs of a new adsorption model in which the adsorption of NH_4^+-N onto biochar is explained by the co-existence of two specific-chemical mechanisms: 1) the CEC and the *ncsf*; 2) other kind of chemical interaction (e.g. hydrogen bonds) responsible of the linear adsorption of NH_4^+-N onto biochar which characterized the last part of the isotherms obtained in Chapter 2 (Section 2.3.3). Biochar surface oxidized over time and negative charges increases (Cheng et al., 2008). For this reason it's possible to suppose that the contribute of the first described mechanism should increase by the time, therefore increasing the final amount of NH_4^+-N adsorbed onto biochar.

4.3 NH_4^+-N adsorption onto biochar is not affected by the temperature neither by the presence of minerals on biochar surface

Specific experiments were conducted to establish whether the adsorption of NH_4^+-N onto biochar is affected by the environmental temperature (Chapter 2) and by the presence of minerals on biochar surface. Fresh biochar is, in fact, characterized by the presence of abundant mineral nutrients on its surface (ashes). Nevertheless the presence of ashes is not a stable characteristic of biochar: in fact, when biochar is buried into the soil, the minerals of its surface are rapidly washed away. By demonstrating that the adsorption of NH_4^+-N onto biochar is dependent on minerals nutrients presence on its surface means that the biochar capability to retain ammonium could be altered in consequence of burying biochar into the soil. A pre-conditioning light acidic treatment was performed onto Euc-350 biochar to wash out the mineral nutrient present on its surface. Batch adsorption experiments conducted at fixed pH conditions revealed that minerals present on biochar surface are not involved on NH_4^+-N adsorption onto biochar. Moreover the NH_4^+-N adsorption onto biochar was not affected either by changes in environmental temperature, thus resulting effective in adsorbing NH_4^+-N in a wide range of temperature (from 20°C up to 55°C). This biochar property could be exploited in area localized at different climatic regions or by adding biochar in composting, during which very high temperature are reached.

4.4 The enrichment of biochar with N fertilizer did not compromise the NUE

Based on the results obtained in this thesis the ‘N equation’ hypothesised by Oenema et al. (2004), according to which in the soil, a high availability of mineral N corresponds to high environmental vulnerability because of more favoured N losses, needs to be revisited when biochar is added to soil. Although biochar demonstrated its ability in retaining NH_4^+-N (Chapter 2), it did not reduced plant NUE (Chapter 3). Biochar resulted in higher NUE compared to other substrate’s amendments, either when it was previously N-charged in N-rich solution, either when the same N fertilizer solution was conventionally applied to the soilless growing media surface (Chapter 3). N fertilization had the higher effect on plant biomass in combination with nutrient rich biochar (e.g. BC1 derived from orchard pruning residues). These results supported the hypothesis of a reversible NH_4^+-N adsorption onto biochar, as already shown by Taghizadeh-Toosi et al. (2012).

Further studies are needed to compare the N-enriched biochar and a BC amended substrate fertilized by widespreading, as usual, the N fertilizer on its surface. Kamman et al. (2011) demonstrated that biochar significantly increased the NUE_{Prod} (aboveground dry matter produced per mg leaf-N) compared to a poor sandy soil medium, in presence of a nutrient balanced solution including micro-nutrients, both in conditions of a lower or of an high water supply. The adequate nutrient content in the growing media, is a prerequisite to observe positive effects of biochar even using nutrient-poor wood-derived biochar (Graber et al., 2010). These evidences lead speculating the potential of biochar to increase the plants yield and the NUE by different mechanisms: (i) the capability to directly provide nutrients (Chapter 3), (ii) the suppression of foliar diseases (Kamman et al., 2011), (iii) the induction of systemic resistence (Graber et al., 2010). The influence of biochar addition on N fate in artificial soilless substrates, remain a big challenge in general, and when the purpose is to optimize the NUE.

4.5 Wood biochar N content is not available for plants

When N was not provided to the growing substrate (Chapter 3) biochar did not affect total yield of *Lolium perenne*. Different biochars independently from their C/N ratios were not able to release their own N. This result confirms the findings of previous studies which observed a low N release from biochar due to the recalcitrant nature of heterocyclic N structure (e.g. Yao et al., 2010; Gaskin et al. 2010; Knicker et al., 2010; Wang et al. 2012). On the other hand, when N was added to the substrate, biochars showed increases of plant productivity in relation with their available nutrients (mostly P and K).

4.6 Biochar as peat substitute in soilless substrates

Biochar role as peat substitute is a major research topic due to the immediate need to avoid peat extraction and exploitation, and to preserve the high ecological value of peatland as wild landscape, wildlife reserve and means for C sequestration (Charman et al., 2009). Biochar demonstrated to be more biologically stable than peat, recalcitrant to degradation, and able to provide nutrients other than N (Chapter 3), with a high exchange capacity (Chapter 2). Moreover biochar demonstrated to be as good as peat in sustaining *Lolium perenne* biomass production and even better than peat when provided with external N inputs. The combination of biochar and N input significantly improved also NUE and water productivity (WP) compared to peat (Chapter 3). Moreover it is known that biochar is a renewable resource, for its nature free from pathogenic organism and it was demonstrated that biochar is good in suppressing soil-borne diseases (Graber et al., 2010). Nevertheless it must be taken into account that not all biochars had the same performance as peat substitute and some of them could be more successful than other (see BC1 and BC2 in Chapter 3) depending, for instance, on the amount of nutrients that they could provide. The benefits of biochar addition to soilless media is also depending on the cropped plant species, and so this factor must be further tested too.

4.7 Future research

Findings presented in this thesis point out toward the high potential of biochar to reduce environmental N losses both to the atmosphere (as $N_{2,}$ N_2O, NH_3. NO_x) and to the ground water (as NO_3, NO_2 or DON). It was studied in detail the NH_4^+-N adsorption mechanisms (Chapter 2), and its effect on plant productivity and N use efficiency (Chapter 3, Section 3.3.2.4).

By using the methodological approach described in Chapter 2 (Section 2.2.2 and 2.2.3) it is possible to predict the quantitative potential of NH_4^+-N adsorption onto biochar, before including it into different matrix as compost, soilless substrates or soil. Biochar specifically designed to retain NH_4^+-N would have immediate benefit to sustainable agricultural practices. It could be exploited to develop new slow release fertilizer, soilless amendment and an additive for compost, with the specific aims to: i) reduce the need of repeated applications of N fertilizer, thus reducing soil compaction and fuel consumption derived from the use of heavy mechanical means; ii) decreasing offsite pollution from composts, meanwhile reducing costs if the N use efficiency of composts is increased; iii) reduce agricultural impacts derived from N losses into the atmosphere and the groundwater.

The use of isotopic technique (^{15}N) is desirable for the future studies in order to quantify the fate of NH_4^+-N adsorbed onto biochar. Further researches must investigate if the use of N-enriched biochar could have effects on: the biochar stability, the native soil organic matter mineralization rates, the reduction of N losses (i.e. maintaining or improving plants NUE).

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