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## Development of a Biochar-Plant-Extract-Based Nitrification Inhibitor and Its Application in Field Conditions

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**Abstract:** The global use of nitrogen (N) fertilizer has increased 10-fold in the last fifty years, resulting in increased N losses via nitrate leaching to groundwater bodies or from gaseous emissions to the atmosphere. One of the biggest problems farmers face in agricultural production systems is the loss of N. In this context, novel biological nitrification inhibitors (BNI) using biochar (BC) as a renewable matrix to increase N use efficiency, by reducing nitrification rates, have been evaluated. The chemical and morphological characteristics of BC were analyzed and BC-BNI complexes were formulated using plant extracts from pine (*Pinus radiata*), eucalyptus (*Eucalyptus globulus*) and peumo (*Cryptocarya alba*). In field experiments, fertilizer and treatments, based on crude plant extracts and BC-BNI complexes, were applied and the effect on nitrification was periodically monitored, and at the laboratory level, a phytotoxicity assay was performed. The

biochar-peumo (BCPe) complex showed the highest nitrification inhibition (66%) on day 60 after application compared with the crude plant extract, suggesting that BCPe complex protects the BNI against biotic or abiotic factors, and therefore BC-BNI complexes could increase the persistence of biological nitrification inhibitors. None of the biochar complexes had toxic effect on radish plants.

**Keywords:** biochar; biological nitrification inhibition; nitrogen

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## 1. Introduction

Rapid population growth has resulted in an increase in food production, resulting in greater consumption of agricultural supplies and nitrogen (N) fertilizer demand [1]. Application of N fertilizer has increased from approximately 10 Tg (1 Tg = 1 million tons) N·year<sup>-1</sup> in the late 1950s to approximately 100 Tg N·year<sup>-1</sup> in 2008. This input plays an important role in the ability of intensive agriculture to maintain high productivity levels [1,2], although N fertilizers also have a large environmental impact [3]. This is due to the dynamic nature of the N cycle, in which nitrification plays a major role [4], transforming agricultural systems into pollution sources. Up to 70% of N-fertilizer input N can be lost via gaseous emissions, as dinitrogen (N<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) or via leaching as nitrate (NO<sub>3</sub><sup>-</sup>) [2]. Through the nitrification process, microorganisms convert NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>, which is highly susceptible to leaching. Nitrate contamination of fresh water systems often triggers eutrophication, altering the equilibrium of aquatic ecosystems [2]. In addition, NO<sub>3</sub><sup>-</sup> can be reduced to nitric oxide (NO) and N<sub>2</sub>O, which contribute to climate change. Agricultural systems worldwide produce nearly 30% of NO and 70% of N<sub>2</sub>O emissions to the atmosphere [5], with the latter a powerful greenhouse gas with a global warming potential approximately 300 times more than CO<sub>2</sub> [1]. Some studies have estimated that global N<sub>2</sub>O emissions by 2100 will be four times greater than the current emissions because of the increasing application of N fertilizers to the soil [5–8]. Therefore, new strategies are required to reduce environmental damage from agricultural N use [3,9]. In this regard, nitrification control could be a strategy to improve N use efficiency (NUE) [4] while also decreasing N<sub>2</sub>O emissions. To improve NUE and to reduce the associated environmental impacts, three methods have been studied in recent years: (a) the use of urease inhibitors that regulate the transformation of organic-N (amide and urine) to ammonia [10]; (b) the use of controlled release fertilizers (CRFs) which have demonstrated many advantages over conventional fertilizers [11,12]; and (c) the use of biological nitrification inhibitors.

Recent studies on mechanisms to improve NUE show that many secondary metabolites produced by plants, such as phenolic acids and terpenoids, can affect the N cycle through the inhibition of the nitrification pathway [13]. Several studies have also reported that some plant species that grow in N-limited soils can produce secondary metabolites that are capable of increasing NUE by inhibiting nitrification, also reducing NO<sub>3</sub><sup>-</sup> loss by leaching [14–16]. These studies evaluated the inhibitory capacity of plant species from a Mediterranean ecosystem by using extracts from the root, bark and leaves of *Cryptocarya alba* and *Eucalyptus globulus* at different doses of application. Results of this study indicated that the bark extract from *E. globulus* at doses of 4 mg·L<sup>-1</sup> showed the highest potential as a nitrification inhibitor. Aqueous leaf extracts from *Quillaja saponaria* and the ethanolic bark extract

from *Pinus radiata* were also effective at inhibiting N mineralization [16]. Previous studies indicate that the bark extract from *E. globulus* and *C. alba* could also inhibit the nitrification process, but the effect was only observed during the first 13 days after the application of N-fertilizer, with no effect after this period [17].

One strategy to increase the efficiency of plant extracts is to incorporate them into organic materials like biochar (BC) [18]. Biochar is produced by pyrolysis of organic material at relatively low temperatures (<700 °C) [19]. Biochar has received considerable agricultural interest as it enhances soil fertility and productivity by increasing cationic exchange capacity (CEC), water retention, aggregation and porosity, soils bioremediation and climate change mitigation [20–22]. The adsorption of plant extracts onto biochar was evaluated in a laboratory experiment, which determined that biochar could be used as a matrix to adsorb organic compounds contained in plant extracts, maintaining its nitrification activity [18]. However, its effectiveness has not yet been evaluated under field conditions. Therefore, the aim of this study was to evaluate the performance of nitrification inhibitors based on biochar plant extract combinations, using bark extracts from *C. alba*, *P. radiata* and *E. globulus* under field conditions.

## 2. Materials and Methods

### 2.1. Selection and Handling of Plant Material and Soil

Plant material was collected from the Mediterranean Area of the south-central zone of Chile (35°58' S and 72°17' W), with an average altitude of 170–180 m a.s.l. and an annual rainfall of 695 mm. The plant extracts used were obtained from bark of the following plant species: pine (*Pinus radiata* D. Don), peumo (*Cryptocarya alba* Mol.) and eucalyptus (*Eucalyptus globulus* Labill). The selected plant extracts used in this study were based on the highest inhibitory effects observed on soil nitrification reported in previous studies [16,17]. The chemical composition of the plant extracts has already been presented [18]. All the plant extracts were obtained with water, except the extract from pine bark, which was obtained using ethanol as solvent, in accordance with the methodology previously described [18]. Separate samples were collected from wood bark of each species, and placed in dark bags to be transported to the laboratory. They were air-dried at the laboratory and extraneous materials were removed. Then, samples were ground using an electric mill (Thomas, Philadelphia, PA, USA), obtaining particles <3 mm.

Plant extracts were obtained using distilled water or ethanol at a ratio of 1:10 weight/volume (dry weight of plant material/volume of solvent). The total solid content was determined gravimetrically by using a known volume of sample that was dried in a porcelain capsule (weighed previously) and then the dry weight of the sample divided by the sample volume to obtain the amount of solid content in the sample. Extracts were stored at 4 °C in amber bottles for a period of up to two weeks.

The laboratory and field experiments were done in the Experimental Station “Los Nogales” of the Universidad de Concepción, Chile, using an Andisol (medial, amorphous, thermic humic haploxerands), with parent material corresponding to modern volcanic ashes and a textural analysis corresponding to silty clay loam (20% sand, 49% lime and 31% clays), having moderate and good drainage [23] containing 7% of organic C and pH of 6. This soil has been used for farming systems because it has high potential for diverse cropping systems (cereals, fruits and prairies), having similar characteristics to other volcanic soils of Central-South of Chile.

## 2.2. Biochar Production

Pieces of pine bark were ground in a mill (Thomas, Philadelphia, PA, USA) to a particle size of 3 mm. A pilot-scale electric pyrolyzer was used to produce biochar. One-kg of material with 51.5% moisture was kept in the pyrolyzer for 15 h with an N-flow rate of  $0.5 \text{ L}\cdot\text{min}^{-1}$ , reaching a maximum temperature of  $550 \text{ }^\circ\text{C}$ , with a residence time of 2 h at the highest temperature.

### Formation of Biochar-Plant Extract Complexes

The previously described methodology [18] was used to sorb the plant extracts onto the biochar surface. Biochar was washed with hot water (at  $100 \text{ }^\circ\text{C}$  during 25 min), then filtered and oven-dried ( $105 \text{ }^\circ\text{C}$ ). Biochar-plant-extract complexes were developed with the goal of maintaining 11.5 mg total solids per gram of biochar. For this, 10 g of dried biochar were placed in high-density polypropylene tubes, and were activated by mixing with a solution of ammonium sulfate at a concentration of  $5 \text{ mg N}\cdot\text{L}^{-1}$  at a ratio of 25:1 (biochar:solution), along with a volume of plant extract equivalent to 11.5 mg total solids per gram of biochar [18]. The procedure of biochar activation was previously tested, using different solvents to activate biochar's surface and to increase the adsorption capacity of phenolic compounds onto biochar (results not shown). Tubes were labeled, wrapped in aluminum foil to prevent exposure to light, incubated at  $25\text{--}30 \text{ }^\circ\text{C}$  and shaken at 150 rpm for 36 h. Complexes and plant extracts are shown in Table 1.

**Table 1.** Identification of the different treatments performed in the field experiments. Asterisk (\*) denotes the treatments that were evaluated in the phytotoxicity assay.

Identification	Description
BC *	Biochar
BCPi *	Biochar with ethanol extract from bark of pine
BCPe *	Biochar with water extract from bark of peumo
BCEu *	Biochar with water extract from bark of eucalyptus
Pine	Ethanol extract from pine
Peumo	Water extract from peumo
Eucalyptus	Water extract from eucalyptus

## 2.3. Chemical Characterization of Biochar

Infrared spectra of the biochar were obtained using FTIR (Bruker, Tensor 27) with the KBr pellet technique. The KBr technique [24] was used to obtain absorption spectra of the organic matter over the range of  $4000$  and  $400 \text{ cm}^{-1}$  wavenumbers. The KBr pellets were prepared by mixing 1 mg of sample with 250 mg KBr (both dried at  $60 \text{ }^\circ\text{C}$  for 36 h) and pressing under a vacuum at  $10,000 \text{ kg cm}^{-2}$ . The resulting pellets were clear and transparent. Two spectra were run for each sample. Infrared spectra were performed at a resolution of  $2 \text{ cm}^{-1}$  and cumulating 16 scans.

#### 2.4. Phytotoxicity Assay

A laboratory experiment was conducted to evaluate the effect of BC and BC-BNI on seed germination of radish (*Raphanus sativus* L.) used as a test plant [25]. The aim of this test was to verify if the treatments represent any risk to the germination of plants. Treatments consisted in BC-BNI (Table 1) at two doses of application, 1 and 2 grams of BC per 100 grams of soil (equivalent to 5 and 10 tons per hectare, respectively). The treatments were mixed with 100 g soil (Andisol; 0–5 cm of depth) as previously described (Section 2.1), and incubated for 15 days at  $25 \pm 2$  °C and 60%–70% of water field capacity, considering a control (with distilled water). Then, the incubated material was filtered at 125 mm diameter (Whatman 42) using distilled water at a ratio of 1:10 (sample:solution), obtaining an extract from each treatment. An aliquot of each extract was used for the germination assay, which consisted of twenty radish seeds (in petri plate) incubated with the extract solutions [25]. After 120 h of incubation in dark conditions at 25 °C, seed germination percentage and root lengths of the plants were determined. Germination indexes (GI) were obtained based on the previous formula [26]. Data were analyzed using ANOVA analysis in a completely randomized design with three replicates per treatment, using SAS statistical software version 8.0.

#### 2.5. Evaluation of Net N Mineralization in a Field Experiment

The net N mineralization assays were established in plots of 0.3 m<sup>2</sup> without plants to prevent alteration of biological processes caused by the interaction of the roots with soil. The application of the BC-BNI was spread onto the soil surface at a dose of 1 g of BC-BNI per 100 g soil (equivalent to 5 tons per hectare), while crude extracts were applied at an equivalent dose to that of BC complexes (11.5 mg total solid of plant extracts per 100 g soil; equivalent to 68 kg·ha<sup>-1</sup>). All treatments (with 3 replicates) received a dose of 85 mg N·kg<sup>-1</sup> soil, equivalent to 100 kg N·ha<sup>-1</sup> using urea.

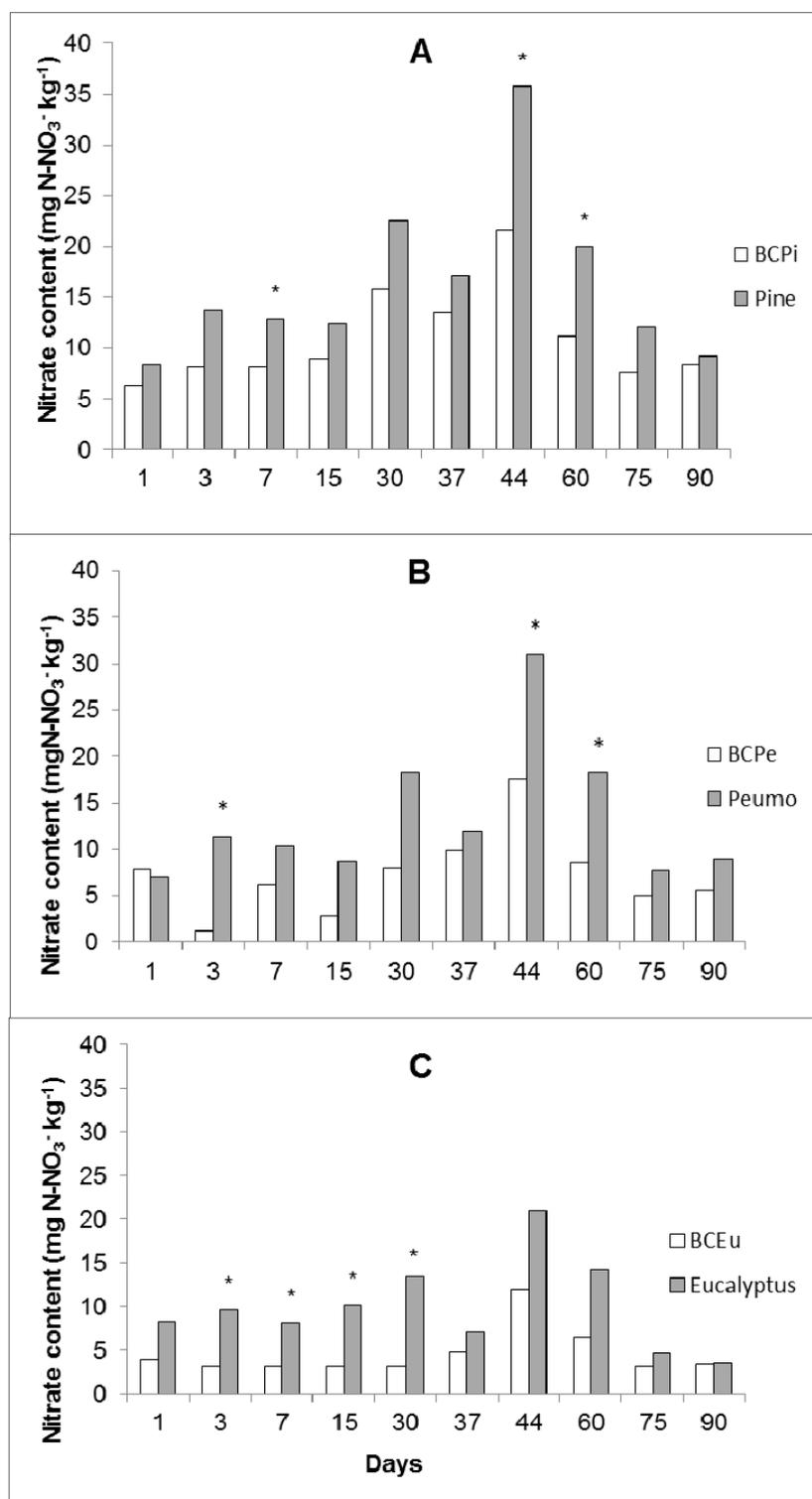
The evaluation of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> produced in the soil was analyzed on days 1, 3, 7, 15, 30, 37, 44, 60, 75 and 90 after N fertilization, collecting soil samples at 0.05 m depth on each sampling date. The NH<sub>4</sub><sup>+</sup> content was determined by the Nessler reaction method [27], while NO<sub>3</sub><sup>-</sup> content was determined using the sulfosalicylic acid method [28]. Results were expressed as mg N (NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>) kg·soil<sup>-1</sup>. The comparisons between the pairwise of treatments (BC-BNI *versus* crude extract) in each sampling date were analyzed with a *t*-test with a 95% confidence interval ( $p \leq 0.05$ ). In order to compare the total amount of NO<sub>3</sub><sup>-</sup> produced during the period, a modified Shapiro-Wilks parametric analysis ( $p \leq 0.05$ ) and Duncan test with a 95% confidence interval ( $p \leq 0.05$ ) were applied. Statistic software INFOSTAT was used to analyze data.

### 3. Results

#### 3.1. Effect of Plant Extracts Adsorbed to Biochar on Nitrification

The effect of BC-BNI *versus* BNI applied as crude extracts is shown in Figure 1. In general, it was found that the BC-BNI complexes had less nitrate produced during the period of field assays. Furthermore, BCPi and BCPe presented statistical differences ( $p \leq 0.05$ ) in the nitrate content until 60 days after N-fertilization *versus* their crude plant extracts (Figure 1A,B) and BCEu until 30 days after

(Figure 1C). When we compared the average of nitrification inhibition of BC-BNI complexes with respect to their crude extracts produced during the entire assay period (at 90 days), values of 59%, 66% and 68% in BCPi, BCPe and BCEu complexes, respectively, were obtained.

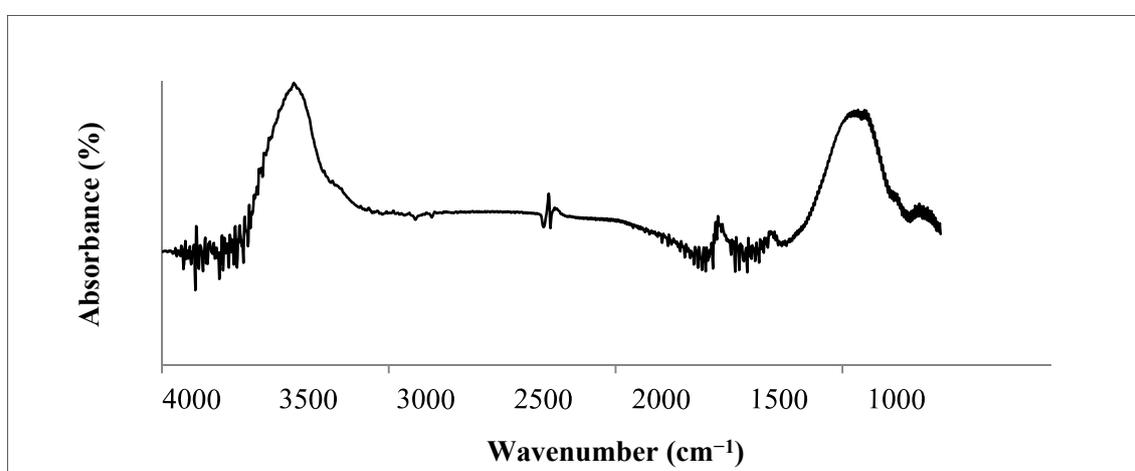


**Figure 1.** Soil nitrate content (mg N-NO<sub>3</sub><sup>-</sup>·kg<sup>-1</sup>) in a field experiment following urea fertilization and the application of (A) BCPi complex and pine extract; (B) BCPe complex and peumo extract; and (C) BCEu complex and eucalyptus extract. Bars with an asterisk (\*) indicate significant differences between treatments (*t*-test, *p* ≤ 0.05).

Afterwards, we determined by comparison between BC-BNI complexes that BCPe was the most effective in decreasing the soil transformation of urea to nitrate, since it presented the lesser amount (Duncan test,  $p \leq 0.05$ ) of nitrate accumulated during the entire period, with  $206 \text{ mg} \cdot \text{NO}_3^- \cdot \text{kg} \cdot \text{soil}^{-1}$  in comparison with BCPi ( $274 \text{ mg} \cdot \text{NO}_3^- \cdot \text{kg} \cdot \text{soil}^{-1}$ ) and BCEu ( $286 \text{ mg} \cdot \text{NO}_3^- \cdot \text{kg} \cdot \text{soil}^{-1}$ ).

### 3.2. Chemical Characteristics of Biochar

FTIR analysis was performed to elucidate the functional groups presented in the chemical structure of the matrix of biochar. The FTIR spectrum of biochar is shown in Figure 2. Different bands in the spectrum represent different vibrations of functional groups. C=C mode was detected at  $1580\text{--}1630 \text{ cm}^{-1}$ , indicating the presence of aromatic rings originating from lignin [29], as condensation of aromatic rings is a consequence of the dehydration of wood heated above  $500 \text{ }^\circ\text{C}$ .



**Figure 2.** Fourier transform infrared spectroscopy (FTIR) spectra of pine bark biochar.

The C=O mode was detected near  $1735 \text{ cm}^{-1}$  and is formed by decomposition of hemicellulose present in the pine bark [29]. The biochar obtained by the carbonization of pine bark at  $550 \text{ }^\circ\text{C}$  resulted in a chemical structure with predominantly aromatic rings that conferred resistance to biological degradation [30]. This indicates that the physical and chemical characteristics of the BC account for the high stability of the matrix [19,31].

### 3.3. Phytotoxicity Assay

The results of the germination index (GI) presented no significant differences between treatments BC-BNI and control ( $p > 0.05$ ) in the germination of radish seeds. The GI in all cases was  $>50\%$ , where this value was used as a limit in bioassays toxicity [32]. There were no negative effects on root elongation (with an average of  $65.26 \pm 8.92 \text{ mm}$ ), which indicate that there were no phytotoxic effects on root development. This suggests that the treatments under study were not toxic.

## 4. Discussion

The inhibitory effect on soil nitrification is attributed to the secondary metabolites present in the plant extracts from pine, peumo and eucalyptus. Tannins are one of the largest groups synthesized by plants.

There is evidence that these compounds influence the N cycle in soil [33] and may alter the amount of inorganic N and soil microorganism activity [34,35].

The crude extracts from bark of peumo and eucalyptus used in this study contained high amount of proanthocyanidins and the crude extract obtained from pine trees contained high levels of epigallocatechins. These are tannins formed by the union of catechins, which in turn are aromatic rings with hydroxyl groups in *para* and *ortho* positions. Such molecular features have a great effect on nitrification inhibition [36].

In our study, the BCPe complex showed the highest inhibitory effect on nitrification compared with other treatments ( $p \leq 0.05$ ), which could be explained by this plant extract presenting a higher content of condensed tannins (1.53 mg catechin equivalent  $\text{mg} \cdot \text{extract}^{-1}$ ) compared to the eucalyptus (0.46 mg catechin equivalent  $\text{mg} \cdot \text{extract}^{-1}$ ) and pine extracts (0.16 mg catechin equivalent  $\text{mg} \cdot \text{extract}^{-1}$ ) [18]. The mode of action of tannins appears to block the ammonia monooxygenase pathway [2,16].

In general, when analyzing  $\text{NO}_3^-$  levels, it is possible to observe that all of the treatments with BC exhibited the highest inhibitory effect on nitrification, indicating a direct effect from BC on the BNI's activity. It has been suggested that organic compounds present in plant extracts are absorbed in the surface area of BC; this is due to the high surface area of BC, ranging between 400 and 2300  $\text{m}^2 \cdot \text{g}^{-1}$  [37–39], which promotes a high adsorption capacity on this matrix. Similarly, based on the extracts' chemical characterizations determined previously [18], the high total phenol content of peumo ( $292.8 \pm 17.1$  mg equivalent of gallic acid  $\text{mg} \cdot \text{extract}^{-1}$ ) in comparison with the other plant extracts suggests that the hydroxyl group of phenols binds to the surface of BC, producing the protection against degradation of this organic molecule and allowing a gradual release of the nitrification inhibitory compounds to the soil even after 60 days of fertilization.

This finding suggests that the mechanisms by which BNI may be preserved over a long period of time include physical protection within micro and nanopores and sorptive protection, acting as a shield for organic compounds against enzymatic attack [40]. Similar results were reported regarding the adsorption of catechol and humic acids from different plant materials in a BC matrix [40]. These authors concluded that BC from pine bark pyrolyzed at 650 °C adsorbed a higher quantity of phenols, coinciding with our study. These physical characteristics promote an effective adsorption of molecules because of the increased surface area and rougher texture of biochar, which improves the adsorption capacity of BC with organic molecules [41–44].

Biochar is a highly stable carbon matrix formed mainly by aromatic rings, characteristics which provide stability to the complex and a high adsorption capacity for organic molecules. It was first proved that biochar-plant-extract complexes did not cause any inhibition or toxicity for the germination of radish seeds and root development.

The biochar-plant-extract complexes based on plant extracts from pine and peumo bark prolonged the inhibitory nitrification effect until 60 days, after application in the field. It is important to note that the peumo biochar extract reached up to 66% nitrification inhibition compared with the crude plant extract. Further research will be focused on product development to enhance the performance of the biochar-plant-extract complexes as nitrification inhibitors.

## 5. Conclusions

The biochar used as matrix to adsorb natural compounds allowed increasing the efficiency of natural extracts; generating a biochar-BNI complex that prolonged the nitrification inhibition at field condition, and consequently could be increase the N use efficiency. The use of this biochar-BNI complex did not produce detrimental effects on test plants; therefore these complexes could be proved in crop systems.

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## Author Contributions

Jhónatan Reyes-Escobar and Cristina Muñoz conceived and designed the experiments and analyzed the data. Erick Zagal, Marco Sandoval and Cristina Muñoz analyzed the data and contributed reagents, materials and analysis tools, and Rodrigo Navia analyzed the data. All authors contributed to the writing of the paper.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

1. Robertson, G.P.; Vitousek, P.M. Nitrogen in agriculture: Balancing the cost of an essential resource. *Annu. Rev. Environ. Resour.* **2009**, *34*, 97–125.
2. Subbarao, G.V.; Sahrawat, K.L.; Nakahara, K.; Ishikawa, T.; Kishii, M.; Rao, I.; Hash, C.T.; George, T.S.; Srinivasa Rao, P.; Nardi, P.; *et al.* Biological Nitrification Inhibition—A Novel Strategy to Regulate Nitrification in Agricultural Systems. *Adv. Agron.* **2012**, *114*, 249–301.
3. Tilman, D.; Cassman, K.G.; Matson, P.A.; Naylor, R.; Polasky, S. Agricultural sustainability and intensive production practices. *Nature* **2002**, *418*, 671–677.
4. Subbarao, G.V.; Ito, O.; Sahrawat, K.L.; Berry, W.L.; Nakahara, K.; Ishikawa, T.; Watanabe, T.; Suenaga, K.; Rondon, M.; Rao, I.M. Scope and strategies for regulation of nitrification in agricultural systems—Challenges and opportunities. *Crit. Rev. Plant Sci.* **2006**, *25*, 303–335.
5. Hofstra, N.; Bouwman, A.F. Denitrification in agricultural soils: Summarizing published data and estimating global annual rates. *Nutr. Cycl. Agroecosyst.* **2005**, *72*, 267–278.
6. Burney, J.A.; Davis, S.J.; Lobell, D.B. Greenhouse gas mitigation by agricultural intensification. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12052–12057.
7. Galloway, J.N.; Townsend, A.R.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.R.; Martinelli, L.A.; Seitzinger, S.P.; Sutton, M.A. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **2008**, *320*, 889–892.

8. Kahrl, F.; Li, Y.; Su, Y.; Tennigkeit, T.; Wilkes, A.; Xu, J. Greenhouse gas emissions from nitrogen fertilizer use in China. *Environ. Sci. Policy* **2010**, *13*, 688–694.
9. Luo, J.; Ledgard, S.F.; Lindsey, S.B. Nitrous oxide and greenhouse gas emissions from grazed pastures as affected by use of nitrification inhibitor and restricted grazing regime. *Sci. Total Environ.* **2013**, *465*, 107–114.
10. Singh, J.; Kunhikrishnan, A.; Bolan, N.S.; Saggar, S. Impact of urease inhibitor on ammonia and nitrous oxide emissions from temperate pasture soil cores receiving urea fertilizer and cattle urine. *Sci. Total Environ.* **2013**, *465*, 56–63.
11. González, M.E.; Cea, M.; Medina, J.; González, A.; Diez, M.C.; Cartes, P.; Monreal, C.; Navia, R. Evaluation of biodegradable polymers as encapsulating agents for the development of a urea controlled-release fertilizer using biochar as support material. *Sci. Total Environ.* **2015**, *505*, 446–453.
12. Guo, M.; Liu, M.; Liang, R.; Niu, A. Granular urea-formaldehyde slow-release fertilizer with superabsorbent and moisture preservation. *J. Appl. Polym. Sci.* **2006**, *99*, 3230–3235.
13. Subbarao, G.V.; Wang, H.Y.; Ito, O.; Nakahara, K.; Berry, W.L.  $\text{NH}_4^+$  triggers the synthesis and release of biological nitrification inhibition compounds in *Brachiaria humidicola* roots. *Plant Soil* **2007**, *290*, 245–257.
14. Castaldi, S.; Carfora, A.; Fiorentino, A.; Natale, A.; Messere, A.; Miglietta, F.; Cotrufo, M.F. Inhibition of net nitrification activity in a Mediterranean woodland: Possible role of chemicals produced by *Arbutus unedo*. *Plant Soil* **2009**, *315*, 273–283.
15. Majumdar, D. Suppression of nitrification and  $\text{N}_2\text{O}$  emission by karanjin—A nitrification inhibitor prepared from karanja (*Pongamia glabra* Vent.). *Chemosphere* **2002**, *47*, 845–850.
16. Suescun, F.; Paulino, L.; Zagal, E.; Ovalle, C.; Muñoz, C. Plant extracts from the Mediterranean zone of Chile potentially affect soil microbial activity related to N transformations: A laboratory experiment. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2012**, *62*, 556–564.
17. Torres, L. *Disminución de las Pérdidas de Nitrógeno Desde un Suelo Andisol Mediante el Uso de Extractos Vegetales. Tesis de Grado*; Universidad de Concepción, Facultad de Agronomía: Chillán, Chile, 2012; p. 47. (In Spanish)
18. Muñoz, C.; Quilodrán, C.; Navia, R. Evaluation of Biochar-Plant Extracts Complexes on Soil Nitrogen Dynamics. *J. Biobased Mater. Bioenergy* **2014**, *8*, 377–385.
19. Lehmann, J.; Joseph, S. *Biochar for Environmental Management: Science and Technology*; Earthscan: New York, NY, USA, **2009**.
20. Lehmann, J.; da Silva, J.P., Jr.; Steiner, C.; Nehls, T.; Zech, W.; Glaser, B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant Soil* **2003**, *249*, 343–357.
21. Lehmann, J.; Gaunt, J.; Rondon, M. Biochar sequestration in terrestrial ecosystems—A review. *Mitig. Adapt. Strategy. Glob. Chang.* **2006**, *11*, 403–427.
22. Woolf, D.; Amonette, J.E.; Street-Perrott, F.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, doi: 10.1038/ncomms1053.
23. Stolpe, N.B. *Descripciones de los Principales Suelos de la VIII Región de Chile*; Departamento de Suelos y Recursos Naturales, Universidad de Concepción: Chillán, Chile, 2006. (In Spanish)

24. Celi, L.; Schnitzer, M.; Nègre, M. Analysis of carboxyl groups in soil humic acids by a wet chemical method, Fourier-transform infrared spectrophotometry, and solution-state carbon-13 nuclear magnetic resonance. A comparative study. *Soil Sci.* **1997**, *162*, 189–197.
25. Sobrero, M.C.; Ronco, A. Ensayo de toxicidad aguda con semillas de lechuga (*Lactuca sativa* L.). In *Ensayos Toxicológicos y Métodos de Evaluación de Calidad de Aguas. Estandarización, Intercalibración, Resultados y Aplicaciones*; Castillo, G., Ed.; Centro Internacional de Investigaciones para el Desarrollo: Ottawa, ON, Canada, 2004; pp. 71–79. (In Spanish)
26. Tiquia, S.M.; Tam, N.F.; Hodgkiss, I.J. Effects of composting on phytotoxicity of spent pig-manure sawdust litter. *Environ. Pollut.* **1996**, *93*, 249–256.
27. Longeri, L.; Etchevers, J.; Venegas, J. Metodología de perfusión para estudios de nitrificación de suelos. *Cienc. Investig. Agrar. (Chile)* **1979**, *4*, 295–299. (In Spanish)
28. Robarge, W.; Edwards, A.; Johnson, B. Water and waste water analysis for nitrate via nitration of salicylic acid 1. *Commun. Soil Sci. Plant Anal.* **1983**, *14*, 1207–1215.
29. Paris, O.; Zollfrank, C.; Zickler, G.A. Decomposition and carbonisation of wood biopolymers—A microstructural study of softwood pyrolysis. *Carbon* **2005**, *43*, 53–66.
30. Baldock, J.A.; Smernik, R.J. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Org. Geochem.* **2002**, *33*, 1093–1109.
31. Schmidt, M.W.I.; Noack, A.G. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Glob. Biogeochem. Cycles* **2000**, *14*, 777–793.
32. Emimo, E.R.; Warman, P.R. Biological assay for compost quality. *Compost Sci. Util.* **2004**, *12*, 342–348.
33. Smolander, A.; Kanerva, S.; Adamczyk, B.; Kitunen, V. Nitrogen transformations in boreal forest soils—Does composition of plant secondary compounds give any explanations? *Plant Soil* **2012**, *350*, 1–26.
34. Hättenschwiler, S.; Vitousek, P.M. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* **2000**, *15*, 238–243.
35. Kraus, T.E.C.; Dahlgren, R.A.; Zasoski, R.J. Tannins in nutrient dynamics of forest ecosystems—A review. *Plant Soil* **2003**, *256*, 41–66.
36. Zhang, L.-L.; Wu, Z.-J.; Shi, Y.-F.; Chen, L.-J.; Song, Y.-C.; Juan, Y.-H. Inhibitory effects of aromatic compounds on soil nitrification. *Pedosphere* **2010**, *20*, 326–333.
37. Downie, A.; Crosky, A.; Munroe, P. Physical properties of biochar. In *Biochar for Environmental Management: Science and Technology*; Earthscan: New York, NY, USA, 2009; Chapter 2, pp. 13–32.
38. Major, J.; Steiner, C.; Downie, A.; Lehmann, J. Biochar effects on nutrient leaching. In *Biochar for Environmental Management: Science and Technology*; Earthscan: New York, NY, USA, 2009; Chapter 15, pp. 271–287.
39. Williams, P.T.; Reed, A.R. Development of activated carbon pore structure via physical and chemical activation of biomass fibre waste. *Biomass Bioenergy* **2006**, *30*, 144–152.
40. Kasozi, G.N.; Zimmerman, A.R.; Nkedi-Kizza, P.; Gao, B. Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). *Environ. Sci. Technol.* **2010**, *44*, 6189–6195.

41. Chen, X.; Chen, G.; Chen, L.; Chen, Y.; Lehmann, J.; McBride, M.B.; Hay, A.G. Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresour. Technol.* **2011**, *102*, 8877–8884.
42. González, M.E; Cea, M.; Sangaletti, N.; González, A.; Toro, C.; Diez, M.C.; Moreno, N.; Querol, X.; Navia, R. Biochar derived from agricultural and forestry residual biomass: Characterization and potential application for enzymes immobilization. *J. Biobased Mater. Bioenergy* **2013**, *7*, 724–732.
43. Zeng, Z.; Zhang, S.-D.; Li, T.-Q.; Zhao, F.-L.; He, Z.-L.; Zhao, H.-P.; Yang, X.-E.; Wang, H.-L.; Zhao, J.; Rafiq, M.T. Sorption of ammonium and phosphate from aqueous solution by biochar derived from phytoremediation plants. *J. Zhejiang Univ. Sci. B* **2013**, *14*, 1152–1161.
44. Zhu, D.; Kwon, S.; Pignatello, J.J. Adsorption of single-ring organic compounds to wood charcoals prepared under different thermochemical conditions. *Environ. Sci. Technol.* **2005**, *39*, 3990–3998.

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