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Cation Exchange Capacity of biochar: an urgent method modification

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17 **Abstract**

18 Biochar, produced through pyrolysis of organic matter, is negatively charged, thus contributing to
19 electrostatic adsorption of cations. However, due to its porous structure and contents of alkaline
20 ashes, the determination of the cation exchange capacity (CEC) is challenging. Literature values
21 for the CEC of biochar are surprisingly variable and are often poorly reproducible, suggesting
22 methodological problems. Here, we modify and critically assess different steps in the existing
23 ammonium acetate (NH_4OAc) method (pH 7), where ammonium (NH_4^+) is displaced by potassium
24 chloride (KCl), following removal of excess NH_4OAc with isopropanol, in batch mode. We used
25 pigeon pea biochar to develop the method and conducted a test on three additional biochars with
26 different acid neutralizing capacity. A pretreatment step of biochar was introduced, using diluted
27 hydrochloric acid, to decrease biochar pH to near neutral, so that 1 M NH_4OAc effectively buffers
28 the biochar suspension pH at 7. This allows the CEC of all biochars to be determined at pH 7,
29 which is crucial for biochar comparison. The dissolution of ashes may cause relatively large weight
30 losses (e.g. for cacao shell biochar), which need to be accounted for when computing the CEC of
31 raw biochar. The sum of NH_4OAc -extractable base cations provided a smaller and better estimate
32 of the CEC than KCl-extractable NH_4^+ . We hypothesize that the overestimation of the CEC based
33 on KCl-extractable NH_4^+ is due to the ineffectiveness of the relatively large isopropanol molecules
34 to remove excess NH_4OAc in biochars rich in micro-pores, due to size exclusion. The amount of
35 base cations removed in the pretreatment was about three (rice husk biochar) to ten times (pigeon
36 pea biochar) greater than the amount of exchangeable cations. The CEC values of biochar
37 increased from 10.8 cmol/Kg carbon to 119.6 cmol/Kg carbon. These values are smaller than
38 reported CEC values of soil organic carbon.

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40 **1. Introduction**

41 Biochar is a carbon-rich product made by pyrolysis of organic waste, which may be used as a soil
42 enhancer. Particularly, in tropical soils biochar has been shown to have a positive impact on soil
43 fertility, including increased potassium (K^+) content, pH, water retention capacity, and cation
44 exchange capacity (Jeffery et al., 2011; Liang et al., 2006; Martinsen et al., 2015), which all
45 contribute to increased crop yield (Jeffery et al., 2017). Moreover, biochar is emerging as an
46 alternative for heavy metal remediation in soil and water (Ahmad et al., 2014). For the assessment
47 of the effect of biochar on soil properties and its potential as remediation agent, it is important to
48 have reliable and accurate determination of its contribution to the soil's cation exchange capacity
49 (CEC). However, in the literature CEC estimates for biochar are highly variable, commonly
50 ranging from 5 to 50 $\text{cmol}_{(+) } \text{Kg}^{-1}$ (Agegnehu et al., 2016; Berek and Hue, 2016; Budai et al., 2014;
51 Gamage et al., 2016; Nelissen et al., 2015; Singh et al., 2010; Song and Guo, 2012) and even
52 reaching values as high as 69 to 204 $\text{cmol}_{(+) } \text{Kg}^{-1}$ (Lou et al., 2016; Mukherjee et al., 2011; Pandit
53 et al., 2018; Yuan et al., 2011). The large variability in CEC may be due to the number of factors
54 affecting the surface properties of biochar, such as charring temperature and feedstock (Budai et
55 al., 2014; Suliman et al., 2016). However, it also may be attributed to errors in the analytical
56 method, which have not yet been properly addressed. Previous research (Graber et al., 2017)
57 summarized the potential sources of error in CEC determination of biochar, which may relate to
58 the presence of ashes, porosity and the intrinsic hydrophobicity of biochar:

- 59 1. Incomplete saturation of exchange sites, due to hydrophobicity of biochar, which may
60 cause poor wetting of the sample and thus underestimation of the CEC.
- 61 2. Slow diffusion of replacing cations, due to the micro-porous structure of biochar, which
62 can prolong the equilibration time, potentially causing underestimation of the CEC.

- 63 3. Floating and non-settling particles can increase substantially the mass losses during the
64 decantation process may cause underestimation of the CEC.
- 65 4. Release of base cations coming from the dissolution of carbonates and silicates present in
66 ashes can interfere with the sum of exchangeable base cations (overestimation of CEC).
- 67 5. Soluble carbonates in biochar can change the pH of the replacing solution, with increasing
68 pH causing an increase in CEC.

69 Methodologies, where displacement after washing is applied are commonly used for CEC analysis
70 in soil (Bache, 1976; Rhoades, 1982) and more recently in biochar (Graber et al., 2017). Often,
71 base cations extracted by NH_4OAc at pH 7 (Chapman, 1965; Hendershot et al., 2008;
72 Schollenberger, 1945) are used to estimate sum of exchangeable base cations, which, in near-
73 neutral soils, often is assumed to be equal to CEC. Alternatively, the CEC is based on displaced
74 NH_4^+ (CEC- NH_4^+) by KCl in a subsequent step, after washing with an organic solvent, such as
75 isopropanol or ethanol. Advantages of NH_4OAc include the avoidance of hydrolysis problems of
76 non-neutral salts, the high wetting and penetration capacity (e.g. compared with barium chloride),
77 and the inexpensive reagents (Schollenberger, 1945). Moreover, NH_4OAc (pH 7) allows
78 comparison of different biochars at neutral pH, which is important, due to the pH dependency of
79 CEC and the high variability in surface properties among different biochars (Mukherjee et al.,
80 2011). However, biochar's CEC may differ considerably between its natural pH and pH 7. In
81 addition, the alkalinity of biochar is highly variable and for some chars it may be needed to adjust
82 pH with acid before using NH_4OAc (pH 7). This may cause problems such as removal of some
83 cations, electrostatically bound to weak acid functional groups at the biochar surface, due to
84 protonation of these groups in response to acid addition, and thus, affecting CEC based on sum of
85 base cations (CEC-BC).

86 Graber et al. (2017) modified a two-step procedure for determination of CEC of biochar using 1M
87 NH_4OAc (pH 7), isopropanol and 2M KCl. In brief, biochar was shaken with 1 M NH_4OAc at pH
88 7 in batch mode and then transferred into a mechanical extractor, where more NH_4OAc was added,
89 before washing with alcohol and subsequently with KCl. According to these authors the method
90 has a number of aspects which need further development, including: 1) The biochar: solution (B:S)
91 ratio required for the saturation with NH_4^+ and displacement with K^+ . 2) The equilibration time of
92 the saturation of exchange sites with NH_4^+ and its subsequent displacement with K^+ . 3) Amount
93 of extraction steps required to get full saturation and subsequent removal of NH_4^+ . In the present
94 study we explore most of these aspects and suggest ways forward. The batch approach ensures
95 contact between the biochar and the solution, which is important when assessing the reaction time
96 with NH_4OAc , KCl and isopropanol and it avoids problems such as preferential flow in the
97 column.

98 In our study we will critically assess (A) the B:S ratio required for the saturation with NH_4^+ and
99 displacement with K^+ , the equilibrium time with 1 M NH_4OAc and 2M KCl, the volume of
100 isopropanol needed to wash the excess NH_4^+ , the optimal volume of 1M NH_4OAc to sufficiently
101 displace cations on biochar surface and the volume of KCl to replace NH_4^+ from the exchange
102 sites, (B) to assess the relative contribution of readily soluble salts vs. exchangeable cations and
103 (C) to compare CEC estimates based on displacement of NH_4^+ by K^+ and CEC based on sum of
104 base cations.

105 **2. Materials and Methods**

106 *2.1 Biochar samples*

107 Four types of biochar were used in this study: pigeon pea biochar (made at 600 °C in an earth-
108 mound kiln at Mkushi, Zambia), cacao shell biochar (made in a locally constructed kiln at Bogor,
109 Indonesia at 350 °C (Hale et al., 2013)), corncob biochar (made in a traditional earth-mound kiln
110 in Zambia at 400 °C during 7 days (Cornelissen et al., 2013)) and rice husk biochar (made at 400-
111 500°C in a drum retort kiln at Chisamba, Zambia at 350 °C (Obia et al., 2016)). The biochar
112 production methods are discussed in the references. In addition, two certified soils materials (NCS
113 DC85101a and NCSDC85113 (NCS, 2017)) were included in the batch experiment. Biochar
114 samples of about 5 L were taken to the Norwegian University of Life Sciences (NMBU), where
115 they were crushed and sieved to 0.5 mm to 2mm.

116 *2.2 Biochar pretreatment*

117 For some of the biochars, the buffered NH₄OAc (pH 7) solution may not adjust the pH to 7 (this
118 is especially important for biochars with high alkalinity). Therefore, CEC estimates with and
119 without pretreatment may diverge, since CEC is pH-dependent. To circumvent this problem, the
120 biochar samples were washed with deionized water and the pH values adjusted to pH 7, using 0.05
121 M hydrochloric acid (HCl), thus removing readily soluble cations associated with salts, ashes and
122 weak acid functional groups. First, one gram of biochar (1.0 ± 0.005) and 20 ml of deionized water
123 were added to 50 ml polypropylene tubes. The tubes were shaken at 200 rpm in a horizontal shaker
124 overnight to ensure a proper wetting of the sample. Next, 0.05 M HCl was gradually added to the
125 tubes until reaching $\text{pH } 7.0 \pm 0.3$. Subsequently, the tubes were shaken during 24 additional hours.
126 The amount of acid added was recorded and the amount of H⁺ to reach pH 7 was calculated to
127 determine the acid neutralizing capacity (ANC_{pH7}). Next, the tubes were centrifuged at 1700g
128 during 15 minutes, the supernatants were removed using a 10 ml pipette and the biochar slurry in
129 the tubes was washed twice more with 20 ml deionized water and shaken for 24 hours each time.

130 The supernatant in the third washing should have EC values $< 0.2 \text{ mS cm}^{-1}$ to avoid significant
131 amounts of base cations in the slurry prior to NH_4OAc addition, which may cause overestimation
132 of exchangeable base cations in the NH_4OAc extracts. The total amount of water and dilute HCl
133 used for each biochar were recorded and the supernatants were stored for analysis of base cations
134 by inductively coupled plasma optical emission spectrometry (ICP-OES).

135 *2.3 Release of exchangeable cations in NH_4OAc (pH 7)*

136 To know the adequate amount of NH_4OAc needed to extract all exchangeable cations, the four
137 types of biochar and two certified soil materials (1 g dry weight of each) were extracted four times
138 consecutively with 20 ml 1M NH_4OAc each time. In the first extraction, the tubes were shaken
139 horizontally during 24 hours at 200rpm, then centrifuged at 1700 g during 15 minutes and the
140 supernatants collected and stored until analysis. In the three subsequent extractions, the
141 supernatants were collected after 2 hours shaking. The extracts were analyzed for Ca^{2+} , Mg^{2+} , K^+ ,
142 Na^+ , Fe, Al and Mn by ICP-OES and the CEC calculated based on sum of exchangeable base
143 cations.

144 *2.4 Washing of excess NH_4OAc with isopropanol and subsequent NH_4^+ extraction with KCl*

145 The adequate amount of isopropanol needed to remove excess NH_4^+ after shaking with NH_4OAc ,
146 was determined, using pigeon pea biochar. The test involved four washings steps with 20 ml 99%
147 isopropanol in triplicate. The biochar was pre-treated as described above; subsequently, 20 ml of
148 1M NH_4OAc was added and the tubes were shaken at 200 rpm during 24 hours. After extraction
149 with NH_4OAc , the biochar slurry was washed with 20 ml 99% isopropanol, once, twice, three and
150 four times, respectively. In each case, 20 ml of 2 M KCl were added subsequently and the tubes
151 were shaken during 24 hours at 200 rpm. The tubes were centrifuged at 1700 g, and the

152 supernatants were analyzed for NH_4^+ (for details see next paragraph). In addition, we determined
153 NH_4^+ in isopropanol extracts after three and four washings in the other biochar types and certified
154 soils.

155 *2.4.1 Biochar to solution ratios and equilibration time for KCl extraction*

156 A factorial 3x3x2 experiment was set up to assess the importance of diffusion of replacing cations
157 (NH_4^+ and K^+) in micro-pores over prolonged time by equilibrating pigeon pea biochar with 1M
158 NH_4OAc (pH7) for 1 and 7 days, respectively, and for 1, 3 and 7 days with 2M KCl. In addition,
159 the effect of three different biochar to solution (B:S) ratios (1:15, 1:30 and 1:45) on the final CEC
160 estimates, based on displaced NH_4^+ in the KCl extract was assessed. One gram of biochar and
161 15ml of NH_4OAc and KCl were used for the treatment 1:15, while 0.5 g and 15ml and 22.5ml
162 were used in the 1:30 and 1:45 treatments, respectively. Only one loading with 1M NH_4OAc and
163 one with 2M KCl was done. The pH adjustment and the washing with water was done in the same
164 way as described above. Every treatment was assessed in triplicates. All the CEC estimates were
165 based on displaced NH_4^+ by K^+ .

166 *2.4.2 Extractions of NH_4^+ by K^+*

167 The amount of 2M KCl needed to displace NH_4^+ by K^+ was determined through three subsequent
168 extractions with 20 ml 2M KCl each time. One gram of the four types of biochar and the two
169 certified soils was weighed in 50 ml plastic tubes. For biochars, the pH and EC adjustment was
170 done as described above. The certified soils were not pretreated. Subsequently, the samples were
171 saturated with NH_4OAc , washed four times with 20ml of 99% isopropanol and then extracted three
172 consecutive times with 2M KCl in triplicates. The CEC was estimated based on displaced NH_4^+ in
173 the KCl extracts.

174 2.5 Chemical analysis

175 The NH_4OAc extracts were analyzed for base cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+) by ICP-OES.
176 Displaced NH_4^+ was analyzed in 2M KCl extracts spectrophotometrically (see SM section). All
177 results were corrected for dry matter content of biochar and background concentration in the
178 sequential extractions. Moreover, the biochars were analyzed for total amount of Ca, Mg, K, Na
179 and after decomposition with nitric acid (HNO_3) and hydrofluoric acid (HF).

180 2.6 Reproducibility

181 We evaluated the reproducibility of the CEC- NH_4^+ estimates by using coefficients of variation
182 (CV), *i.e.* the relative standard deviation, of those experiments in which we carried out 3 and 4
183 washings with isopropanol. For CEC-BC we used CV of experiment 2 (Table 1), in which four
184 consecutive extractions with 1M NH_4OAc (pH 7) were done.

185 2.7 Mass loss

186 After the extraction with 2M KCl, the biochar slurries were washed 3 times with 35ml deionized
187 water to remove the excess of salt, until $\text{EC} \approx 0.4 \text{ mS cm}^{-1}$ and dried during a week at 70 °C. The
188 mass loss during all the procedure was determined and the CEC values referred to the initial raw
189 biochar, corrected for dry matter content.

190 2.8 Statistical analysis

191 Statistical analysis of the experiment described in section 2.6 was done using the R software (R-
192 Core-Team, 2017). The CEC estimates of the samples shaken for 1 and 7 days with NH_4OAc , 1,
193 3 and 7 days with 2M KCl and the three different B:S were subjected to one-way ANOVA.

Experiment	Type of biochar	Objective	Description/Treatments
1. Biochar pretreatment	Pigeon pea, rice husk, cacao shell and corncob	To Remove acid-soluble salts and adjust pH	pH adjustment to 7 and washing of samples until $EC < 0.2 \text{ mS.cm}^{-1}$
2. Release of exchangeable cations in NH_4OAc (pH7)	Pigeon pea, rice husk, cacao shell and corncob	To know the amount of NH_4OAc needed to exchange the base cations	Four consecutive extractions with 20ml NH_4OAc in each extraction.
3. Washing of excess NH_4OAc with isopropanol and subsequent NH_4^+ extraction with KCl	1st part: Pigeon pea 2nd part: Pigeon pea, rice husk, cacao shell and corncob	To know the right amount of isopropanol needed to remove the excess NH_4^+	1st part: 1, 2 and 3 washings with 20ml isopropanol each time. Analysis of NH_4^+ in KCl extracts of pigeon pea biochar. 2nd part: analysis of NH_4^+ in isopropanol extracts in the 3 rd and 4 th washing for all the biochars.
3.1 Biochar to solution ratios and equilibration time for KCl extraction	Pigeon pea	To assess the diffusion of NH_4^+ and K^+ in micro-pores and the effect of three different B:S on the CEC estimates, based on NH_4^+ in KCl extract	B:S : 1:15/1:30/1:45 NH_4OAc: 1 and 2 days KCl: 1, 3 and 7 days
3.2 Extractions of NH_4^+ by K^+	Pigeon pea, rice husk, cacao shell and corncob	To know the amount of KCl needed to fully displace NH_4^+	Three consecutive extractions with 20ml KCl in each extraction

196 **3. Results and discussion**

197 *3.1 Pretreatment*

198 The ANC_{pH7} was largest in cacao shell biochar followed by that of pigeon pea biochar despite the
199 higher pH of the latter (Table 2). Rice husk and corncob biochar had a relatively small ANC_{pH7} ,
200 although also these biochars had $pH > 8.5$. In accordance with this, the cumulative amount of base
201 cations (expressed in $cmol_{(+)} / Kg$) removed in three consecutive washings and the initial EC
202 decreased in the same order with cacao shell biochar $>$ pigeon pea biochar $>$ corncob biochar $>$
203 rice husk biochar (Table 2). In the third washing of the pretreatment the removal of base cations
204 declined to only $7.5 cmol_{(+)} Kg^{-1}$ (cacao shell biochar), $2 cmol_{(+)} Kg^{-1}$ (pigeon pea biochar), 1.3
205 $cmol_{(+)} Kg^{-1}$ (corncob biochar), $1 cmol_{(+)} Kg^{-1}$ (rice husk biochar), thus contributing little to
206 ANC_{pH7} . The cumulative amount of removed cations in the three washings exceeded ANC_{pH7}
207 (Table 2) indicating that a considerable proportion of the extracted cations are due to dissolution
208 of salts (47 to 71%, except pigeon pea biochar for which this was 14%).

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216 **Table 2.** pH, electrical conductivity (EC), acid neutralizing capacity (ANC_{pH7}) and the
 217 accumulated sum of base cations (Ca, Mg, K, Na) removed during the three washings of the
 218 pretreatment of biochar with water and acid. The first washing was done with water and acid
 219 followed by two consecutive washings with 20ml deionized water each time. The values are
 220 referred to raw biochar.

	¹ pH	<i>s.d</i>	² EC	<i>s.d</i>	ANC _{pH7}	1 st	2 nd	3 rd	³ Δ
			mS cm ⁻¹		cmol ₍₊₎ Kg ⁻¹	cmol ₍₊₎ Kg ⁻¹	cmol ₍₊₎ Kg ⁻¹	cmol ₍₊₎ Kg ⁻¹	cmol ₍₊₎ Kg ⁻¹
Pigeon Pea	10.4	0.03	1.4	0.02	49	47.2 ± 0.3	7.4 ± 0.2	2.0 ± 0.07	7.6
Cacao Shell	9.6	0.03	8.9	0.18	134	219 ± 5.0	24.5 ± 0.4	7.5 ± 0.4	117
Corncob	8.5	0.04	0.8	0.01	7	15.7 ± 0.4	3.4 ± 0.2	1.3 ± 0.09	13.4
Rice husk	8.7	0.05	0.4	0.01	4	10.1 ± 0.2	2.7 ± 0.3	1.0 ± 0.05	9.8

221 ¹ Measured in 1:5 deionized water and raw biochar.

222 ² Measured in 1:20 deionized water using raw biochar.

223 ³ Difference between accumulated sum of cations removed in the pretreatment and ANC_{pH7}

224 3.2.1 Release of exchangeable cations in the NH₄OAc (pH 7) extract

225 On average, 83% of all exchangeable base cations of the biochar samples were extracted in the
 226 first extraction with 20 ml NH₄OAc (Table 3). Both certified soils had very similar patterns as
 227 pigeon pea and rice husk BC, with 88% of the base cations removed in the first extraction. After
 228 four extractions, the sum of exchangeable base cations decreased in the order cacao shell biochar
 229 > pigeon pea biochar > rice husk biochar > corncob biochar (Table 3). The average sum of
 230 exchangeable base cations of the certified soils was 20.7 and 26.9 cmol₍₊₎ Kg⁻¹ for DC85101a and

231 DC85113, respectively. The certified values of sum of exchangeable cations for these soils are
 232 23.4±2.4 and 29±1.4 cmol₍₊₎/Kg, respectively (NCS, 2017).

233 **Table 3.** Percentage of exchangeable base cations extracted in four consecutive extractions with
 234 20ml 1M NH₄OAc (pH 7) for four types of biochars and two certified soils. CEC values are
 235 referred to raw biochar.

	CEC-BC	<i>s.d</i>	First	<i>s.d</i>	Second	<i>s.d</i>	Third	<i>s.d</i>	Fourth	<i>s.d</i>
	cmol₍₊₎ Kg⁻¹		(%)		(%)		(%)		(%)	
Pigeon Pea	6.6	1.6	77.5	0.3	13.6	0.3	5.9	0.2	3.0	0.1
Cacao Shell	59.1	4.2	75.6	2.5	12.2	1.0	8.4	0.7	3.8	0.8
Corncob	6.0	1.8	90.1	1.1	5.2	1.2	3.0	0.2	1.7	0.1
Rice husk	6.2	0.03	87.7	1.4	7.8	1.2	2.9	0.3	1.5	0.1
DC85101a	20.7	0.5	88.5	0.4	7.6	0.2	2.8	0.1	1.2	0.2
DC85113	26.9	0.5	87.6	0.7	8.8	0.7	2.6	0.1	1.1	0.0

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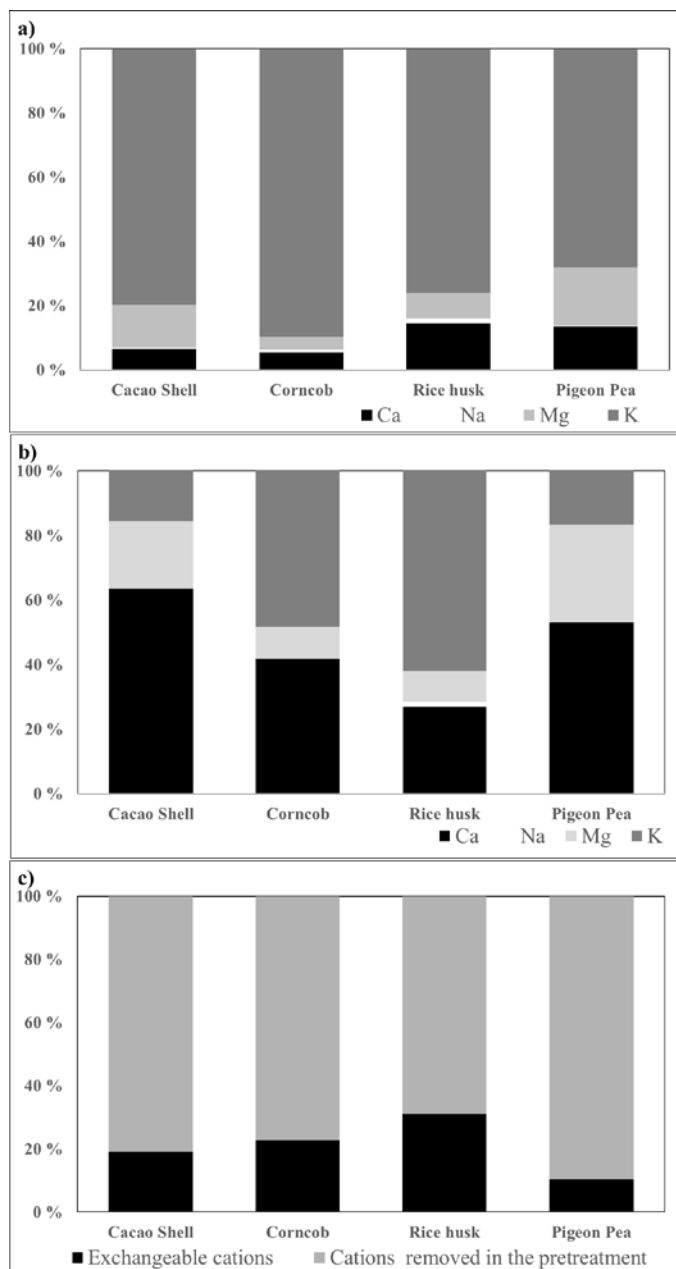
237 Among the exchangeable base cations, Ca²⁺ is the most abundant in cacao shell and pigeon pea
 238 biochars (63 and 53 % of the total respectively), followed by Mg²⁺ (20.8 and 30%) K⁺ (15.5 and
 239 16.6%) and Na⁺ (0.15 and 0.14%) (Figure 1 b). In corncob and rice husk biochars, K⁺ was the most
 240 abundant (48 and 63% respectively), followed by Ca²⁺ (41 and 27%) Mg²⁺ (10 and 9.6 %) and Na⁺
 241 (0.2 and 0.9%). This difference in Ca²⁺ and K⁺ saturation is related to the type of feedstock, with
 242 grass-type feedstocks being richer in K⁺ than the other biochars used in this study. In both soil
 243 samples Ca²⁺ represented about 70% of the total exchangeable cations, whereas this was about
 244 24%, 3% and 1% for Mg²⁺, K⁺, and Na⁺, respectively. As expected at high pH, exchangeable Fe,
 245 Al and Mn in the biochars were small (Table S1).

246 3.2.2 Cations removed in the pretreatment vs exchangeable cations

247 The amount of cations removed during the pretreatment was higher than the amount of
248 exchangeable cations for all the biochars (Table S2). When combining the exchangeable cations
249 and the cations removed in the pretreatment, the exchangeable fraction accounts for less than 31%
250 (Figure 1c.) with the highest values observed for rice husk biochar (31%), followed by corncob
251 (22.7%), cacao shell (19%) and pigeon pea (10.4%) biochar. The proportion of individual base
252 cations relative to total exchangeable cations differed from that observed in the pretreatment
253 (Figure 1 a. and b.). In general, K^+ is the most abundant base cation removed in the pretreatment
254 ($> 68\%$) for all the biochars (Figure 1a), while Ca^{2+} was more important in the exchangeable
255 fraction, particularly for pigeon pea and cacao shell biochars as indicated above (Figure 1b). The
256 proportion of Mg^{2+} to the total readily soluble cations was 3.9% for corncob, 7.9% for rice husk,
257 13.3% for cacao shell and 18.2% for pigeon pea biochar. In general, Mg^{2+} is more abundant in the
258 exchangeable fraction of cations than in the readily soluble ones. The contribution of Na^+ to the
259 exchangeable fractions and the readily soluble cations was minor, being the highest for rice husk
260 biochar with 1.6% of the exchangeable cations and 1.4% of the cations removed in the
261 pretreatment.

262 By pretreating biochar, we washed away the readily soluble cations, associated with ashes and
263 salts, which otherwise would have dissolved in NH_4OAc (pH 7). The amount of base cations
264 removed in the third washing of the pretreatment (Table 2) was considerably lower than CEC-BC
265 (Table 3). This was the reason for setting 0.2 mS cm^{-1} as EC threshold in the pretreatment. Previous
266 research (Martinsen et al., 2015) using a sub sample of the same cacao shell biochar showed that
267 CEC-BC without any pretreatment was $197 \text{ cmol}_{(+)}/\text{Kg}$, while in our experiment this was 59.1
268 $\text{cmol}_{(+)}/\text{Kg}^{-1}$ with pretreatment. Furthermore, Graber et al. (2017) found significant differences
269 between CEC-BC and CEC- NH_4^+ for some of the biochars they analyzed. Without pretreatment,

270 they found that for wheat straw biochar made at 550 °C and at 700 °C, the sum of base cations in
 271 NH₄OAc was 4 and 5 times greater than CEC-NH₄⁺. Therefore, when the sum of exchangeable
 272 cations is used to estimate CEC, a pretreatment removing readily soluble salts is compulsory, to
 273 prevent the contribution of soluble components of ashes, especially for biochars with high EC.



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275 Figure1. a) Contribution of individual cations to the total amount readily soluble cations removed
276 in the pretreatment, b) Contribution of individual cations to the total amount of exchangeable
277 cations. c) Relative contribution of cations removed during the pretreatment and the exchangeable
278 cations.

279 *3.3 Washing of excess NH₄OAc with isopropanol and subsequent NH₄⁺ extraction with KCl*

280 The 2M KCl extractable NH₄⁺ significantly decreased upon washing with isopropanol with a
281 reduction of 66% from the first to the third washing (Figure S 1). The analysis of NH₄⁺ in
282 isopropanol extracts after washing three and four times revealed small amounts of excess NH₄⁺ for
283 all biochars and soils (Table S3).

284 *3.3.1 Biochar to solution ratios and equilibration time for KCl extraction*

285 There were no significant ($p = 0.64$, Table S4) differences in the CEC estimates of pigeon pea
286 biochar based on KCl extractable NH₄⁺, if the biochar had been shaken with NH₄OAc for 1 or 7
287 days (27.3 (± 2.3) cmol₍₊₎ Kg⁻¹ and 27.0 (± 2.4) cmol₍₊₎/Kg, respectively). This indicates that in there
288 was no diffusion limitation to saturate biochar with NH₄⁺ despite the presence of micro-pores that
289 could restrict the entrance of NH₄⁺ ions. In addition, there was no significant ($p = 0.88$, Table S4)
290 difference in CEC estimates of pigeon pea biochar if shaken with 2M KCl, for 1, 3 or 7 days. This
291 indicates that within one day there is full displacement of NH₄⁺. Moreover, there were no
292 significant differences between the three B:S ratios evaluated ($p = 0.54$, Table S4). The average
293 CEC values were 26.6 (± 2.2) cmol₍₊₎/Kg, 27.4 (± 1.6) cmol₍₊₎ Kg⁻¹ and 27.4 (± 3.0) cmol₍₊₎ Kg⁻¹ for
294 1:15, 1:30 and 1:45 treatments, respectively. In all cases, the B:S ratio was enough to fully saturate
295 the exchange sites. To have ample solution volume for chemical analysis of the extracts, we used
296 20 ml of 1M NH₄OAc and 2M KCl.

297 3.3.2 Extractions of NH_4^+ by K^+

298 CEC- NH_4^+ decreased in the order cacao shell biochar > rice husk biochar > pigeon pea biochar >
 299 corncob biochar (Table 4). The first extraction with 20 ml 2M KCl following isopropanol washing
 300 removed 93% of the adsorbed NH_4^+ for all the biochars (Table 4) and 98.7% for the certified soils.
 301 The certified CEC values for soils are 21.6 ± 1.6 and 31 ± 1 $cmol_{(+)} Kg^{-1}$; ((NCS, 2017); note that
 302 the certified CEC values are different than sum of exchangeable base cations) and our results
 303 summing the three consecutive extractions were 23.8 and 34 $cmol_{(+)} Kg^{-1}$ for DC85101a and
 304 DC85113. However if we only consider the value from the first extraction with 2M KCl, the CEC
 305 values fit in the certified intervals. This indicates that at least one extraction with 20ml 2M KCl is
 306 enough to have a reasonable estimate of CEC- NH_4^+ values of biochar and soil samples.

307 **Table 4.** CEC based on displaced NH_4^+ (CEC- NH_4^+) and percentage of NH_4^+ extracted in 3
 308 consecutive extractions with 2M KCl. CEC values are referred to raw biochar.

Sample type	CEC- NH_4^+		First KCl	Second KCl	Third KCl
	$cmol_{(+)}/Kg$	C.V (%)	(%)	(%)	(%)
Pigeon Pea	26,7	8.4	93.5	4.5	2.0
Cacao Shell	59.7	2	93.4	4.2	2.4
Corn cob	19.1	4.6	96.3	1.3	2.5
Rice husk	27,3	1.2	89.1	7.7	3.2
Soil-DC85101a	23.8	0.25	99.3	0.4	0.3
Soil-DC85113	34.0	1.3	98.1	1.6	0.3

309

310

311 3.4 Mass loss

312 On average, the mass loss of the raw biochars during the batch procedure was 26%, 1.7%, 1.6%
313 and 10% of the raw biochar for cacao shell biochar, corncob biochar, rice husk biochar and pigeon
314 pea biochar, respectively. The pretreatment data indicate that a significant amount of Ca, Mg and
315 K was lost as salts and also through dissolution of carbonates and silicates (included in ANC_{pH7}).
316 Based on the removal of Ca, Mg, K and Na in the pretreatment we estimated a weight loss of 8.6%,
317 0.7%, 0.5 % and 1.8% for cacao shell, corncob, rice husk and pigeon pea biochars, respectively.
318 Presumably the loss is at least twice as much, because the dissolution of cations is coupled with
319 the dissolution of anions like chlorides, sulfates, carbonates and silicates. Based on this it is likely
320 that nearly all mass loss is due to the removal of salts, carbonates and silicates in the pretreatment,
321 whereas the loss of pure biochar was limited.

322 During the pretreatment, the supernatant of corncob biochar was the only with dark color (Figure
323 S2), however, the mass loss of this biochar was very low. Probably the loss of pure biochar was
324 small, as the extracts were carefully pipetted instead of decanted and because the ionic strength of
325 the extracting solutions caused flocculation and settling of the brown colored colloids of the
326 biochar suspensions. In addition, three additional washings with water were done after the
327 extraction with 2M KCl in order to remove the excess of salt and to estimate the total mass loss.
328 Thus, the total mass loss during the batch procedure is an overestimation.

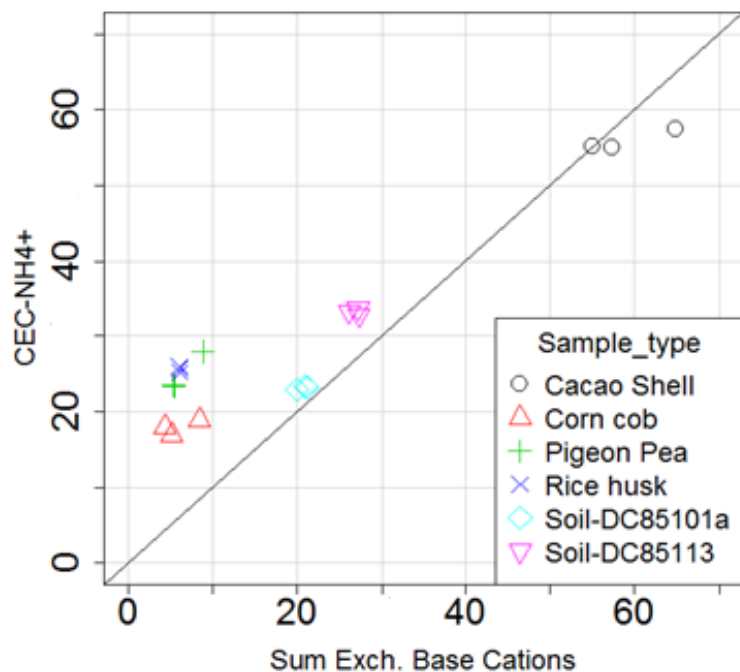
329 3.5 Comparison of CEC-BC and CEC-NH₄⁺ for biochar

330 For cacao shell biochar and the two certified soils, CEC-NH₄⁺ to CEC-BC ratios were 1.0, 1.1 and
331 1.2 respectively, indicating reasonable correspondence between both methods (Figure 2).
332 However, for the other biochars, CEC-NH₄⁺ was 3.9, 4.1 and 3.2 times greater than CEC-BC

333 (Figure 2). A likely explanation is that these biochars are rich in micro-pores (not analyzed) and
334 isopropanol does not effectively remove excess NH_4^+ trapped in the micro-pores, presumably
335 because of size exclusion of isopropanol molecules. Exclusion of organic molecules from small
336 pores due to steric effects was reported earlier for wood charcoals (Zhu et al., 2005). Previous
337 research (Pignatello et al., 2006) described a molecular sieving effect in which large natural
338 organic compounds were more restricted to the external sites of environmental black carbon and
339 thus, blocking the entrance of N_2 to the interior of the narrowest pores when analyzing surface
340 properties. Moreover, previous research indicated that biomass-based activated carbon was
341 dominated by pores in the size range of 3.5 – 15 Å (Amstaetter et al., 2012). Also torrefied maize
342 and cotton stalks made at 290°C had a significant amount of pores between 4 and 5 Å (Chen et al.,
343 2014). Considering that the molecular diameter of isopropanol is 5.2 Å (Van der Bruggen et al.,
344 1999), it is likely that it cannot penetrate the smallest pores, thus reducing its effectiveness to
345 remove excess NH_4OAc . Therefore, we recommend to wash excess NH_4OAc in biochar with
346 solvents having smaller molecular size than isopropanol, e.g. ethanol or even water. To our
347 knowledge there is no study of the effectiveness of different solvents to remove excess index
348 cations in CEC methodologies for biochar. Due to the limitations of using isopropanol as a washing
349 agent, we believe that CEC-BC after pretreatment of biochar provides a better estimate of its CEC
350 than CEC- NH_4^+ .

351 The CEC- BC was 119.6 $\text{cmol}_{(+)}\text{Kg C}^{-1}$, 11.6 $\text{cmol}_{(+)}\text{Kg C}^{-1}$, 10.8 $\text{cmol}_{(+)}\text{Kg C}^{-1}$, and 14.1 $\text{cmol}_{(+)}$
352 Kg C^{-1} for cacao shell biochar, pigeon pea biochar, corncob biochar and rice husk biochar,
353 respectively. These values are smaller compared to those reported for soil organic C, (221-330
354 $\text{cmol}_{(+)}\text{Kg C}^{-1}$ (Parfitt et al., 2008); 370-500 $\text{cmol}_{(+)}\text{Kg C}^{-1}$ (Gruba and Mulder, 2015) ;540-810
355 $\text{cmol}_{(+)}\text{Kg C}^{-1}$ (Martinsen et al., 2017)) and closer to what other studies have published for biochar

356 (75 cmol₍₊₎ Kg C⁻¹, (Silber et al., 2010)). Total organic carbon of the biochars are given in Table
357 S5.



358

359 **Figure 2.** CEC-NH₄⁺ (cmol₍₊₎/Kg) as function of CEC-BC (cmol₍₊₎/Kg;). The line is the 1:1 line.

360 3.6 Reproducibility

361 The reproducibility of CEC-BC was good for the biochars of rice husk and cacao shell, and rice
362 husk biochar, but less so for corncob and pigeon pea biochars (Table 5). Probably, the CV values
363 for CEC-BC were relatively high, because they are a summation of four cations, each adding to
364 the uncertainty of the sum. Although, representing an overestimation, due to ineffective washing
365 of excess NH₄OAc, the reproducibility of CEC-NH₄⁺ was superior and if a more appropriate
366 solvent is found it is to be preferred over CEC-BC. Even if CEC-NH₄⁺ estimates with 3 and 4
367 washings with isopropanol were bulked (Table 5), CV was lower than 10% for cacao shell, pigeon
368 pea biochar and rice husk biochar, while this was slightly higher for corncob biochar (14.5%, Table

369 5). CV values of CEC-NH₄⁺ may be decreased further for all the biochars if only the values from
 370 the experiment with four isopropanol washings are included (Table 4). The experiments with 3
 371 and 4 washings with isopropanol were combined because there were only three replicates using 4
 372 washings with isopropanol.

373 **Table 5.** CV of CEC-NH₄⁺ combining all the experiments in which 3 and 4 washing with
 374 isopropanol were done and CV of CEC-BC of the experiment with four consecutive extractions
 375 with 1M NH₄OAc.

Feedstock	CEC-NH ₄ ⁺				CEC-BC	
	Replicates	CEC-NH ₄ ⁺ (cmol(+)/kg)	Sd	CV (%)	Replicates	CV (%)
Pigeon Pea	60	27.1	2.3	8.4	3	24.4
Cacao Shell	11	58.1	4.4	7.5	3	7.1
Corn cob	11	22.4	3.3	14.5	3	29.5
Rice husk	11	26.7	0.8	3.0	3	0.4

377 4. Conclusions

378 The NH₄OAc (pH 7) method for CEC determination based on displacement of NH₄⁺ by KCl was
 379 tested for biochar in batch mode and compared with CEC values obtained from the sum of
 380 extractable cations in NH₄OAc.

381 A compulsory pretreatment was shown to be necessary to remove readily soluble cations from
 382 salts, carbonates and silicates and adjust biochar pH to near neutral. The amount of readily soluble
 383 cations removed in the pretreatment was several times greater than the exchangeable fraction for
 384 all biochars. Skipping the pretreatment causes a major overestimation of the CEC of biochar.
 385 Pretreatment of biochar also causes major mass losses, due to the removal of salts, carbonates and
 386 silicates. These mass losses have to be quantified in order to be able to compute the CEC of the
 387 initial raw biochar. The most abundant cation in the readily soluble fraction of the pretreatment of

388 all biochars was K^+ . The exchangeable cations (CEC-BC) were dominated by K^+ for rice husk
389 and corncob biochars, whereas this was Ca^{2+} for pigeon pea and cacao shell biochars.

390 This study shows that shaking 1 g of biochar, following pretreatment, with 1M NH_4OAc during
391 24 hours was enough to saturate the exchange sites with NH_4^+ . Additional 1M NH_4OAc was
392 needed to fully extract the exchangeable cations. There was no additional NH_4OAc removed after
393 four consecutive washings with 20ml isopropanol. However our results suggest that isopropanol
394 may not penetrate the smallest pores of some biochars and we conclude that other liquid
395 compounds are required to fully remove excess NH_4OAc . Shaking with 2M KCl during 24 hours
396 fully displaced NH_4^+ from the exchange sites. The biochar to solution ratios did not affect the CEC
397 estimates.

398 CEC- NH_4^+ , although representing an overestimation due to the inefficient removal of excess
399 NH_4OAc by isopropanol, was more reproducible than CEC-BC.

400 A detailed description of the protocol for the modified method in batch is presented in the SM.

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