

1 Phosphorus fertilisation under nitrogen limitation can deplete soil  
2 carbon stocks - Evidence from Swedish meta-replicated long-term  
3 field experiments

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## 20 Abstract

21 Increasing soil organic carbon (SOC) in agricultural soils can mitigate atmospheric CO<sub>2</sub>  
22 concentration and also contribute to increase soil fertility and ecosystem resilience. The role of  
23 major nutrients on SOC dynamics is complex, due to simultaneous effects on net primary  
24 productivity (NPP) that influence crop residue carbon inputs and on the rate of heterotrophic  
25 respiration (carbon outputs). This study investigated the effect on SOC stocks of three different  
26 levels of phosphorus and potassium (PK) fertilisation rates in the absence of nitrogen fertilisation  
27 and of three different levels of nitrogen in the absence of PK. This was done by analysing data  
28 from 10 meta-replicated Swedish long-term field experiments (>45 years). With N fertilisation,  
29 SOC stocks followed yield increases. However, for all PK levels, we found average SOC losses  
30 ranging from  $-0.04 \pm 0.09 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  (ns) for the lowest to  $-0.09 \pm 0.07 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  ( $p=0.008$ ) for  
31 the highest application rate, while crop yields as a proxy for carbon input increased significantly  
32 with PK fertilization by 1, 10 and 15%. We conclude that SOC dynamics are mainly output-  
33 driven in the PK fertilised regime but mostly input-driven in the N fertilised regime, due to the  
34 much more pronounced response of NPP to N than to PK fertilisation. It has been established  
35 that P rather than K is the element affecting ecosystem carbon fluxes, where P fertilisation has  
36 been shown to: i) stimulate heterotrophic respiration, ii) reduce the abundance of arbuscular  
37 mycorrhizal fungi and iii) decrease crop root:shoot ratio, leading to higher root-derived carbon  
38 input. The higher export of N in the PK fertilised plots in this study could iv) have led to  
39 increased N mining and thus mineralisation of organic matter. More integrated experiments are  
40 needed to gain a better understanding of the relative importance of each of the above-mentioned  
41 mechanisms leading to SOC losses after P addition.

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## 43 1. Introduction

44 Maintaining or increasing soil organic carbon (SOC) stocks in agricultural soil is important for  
45 ecosystem resilience, soil fertility and sustainable crop production. Sequestration of SOC is also  
46 an important climate mitigation option (Lal, 2003; Lugato et al., 2006). The annual export of  
47 plant biomass demands a sufficient nutrient supply, mainly of nitrogen (N), phosphorus (P) and  
48 potassium (K). To cope with steadily increasing global food demand and limitations to spatial  
49 expansion of agricultural area, the optimal balance between production and sustaining soil  
50 fertility has to be found. The magnitude and dynamics of SOC stocks are determined by the ratio  
51 between inputs and outputs of carbon. It is a well-established fact that mineral (NPK) fertiliser  
52 has a strong positive effect on net primary production (NPP) and thus on carbon inputs (Kätterer  
53 et al. 2012). In three global meta-analyses, the increase in topsoil SOC following mineral  
54 nitrogen fertilisation was reported to range from 3.5 to 8% (Alvarez, 2005; Ladha et al., 2011; Lu  
55 et al., 2011). Mineral fertilisation is therefore generally recommended as a suitable management  
56 practice for SOC sequestration (Lugato et al., 2006). In many long-term experiments with  
57 differing fertiliser treatments, SOC stocks have been well explained by measured or estimated  
58 NPP (Kätterer et al., 2012). However, the effect of N fertilisation on the corresponding C output  
59 by heterotrophic respiration and the mechanisms involved are still not fully understood. Nitrogen  
60 fertilisation has been shown to decrease respiratory C losses, especially in combination with a  
61 labile C source amendment. This is mostly explained by the “N mining”-theory, which predicts  
62 accelerated microbial decomposition of more recalcitrant, energy-poor organic matter in the  
63 search of nitrogen under N deficiency. Energy for this break-down is thereby derived from labile  
64 C, which in turn cannot efficiently be used for biosynthesis (Bradford et al., 2008; Craine et al.,  
65 2007; Milcu et al., 2011; Schimel and Weintraub, 2003). Cleveland and Liptzin (2007) reported a

66 globally well constrained microbial biomass C:N:P ratio of 60:7:1, which indicates  
67 stoichiometric constraints for microbial growth. Recently, Murphy et al. (2015) observed that  
68 specific N-rich compounds of soil organic matter were primed after glucose addition. This  
69 observation can be interpreted as selective N-mining. The opposite effect, i.e. acceleration of C  
70 mineralisation by N addition, has also been reported (Allen and Schlesinger, 2004; Milcu et al.,  
71 2011; Reed et al., 2011), indicating that N can be a limiting factor for microbial breakdown. To  
72 date, studies about the effect of mineral fertiliser on SOC have mainly focused on N or combined  
73 N, P and K fertiliser, because nitrogen has the most obvious effects on the carbon cycle and NPK  
74 is the most common fertiliser combination used in commercial agriculture. However, it has  
75 recently been shown that P or PK fertilisation can have stimulating effects on heterotrophic  
76 respiration (Cleveland and Townsend, 2006; Cleveland et al., 2002; Fisk et al., 2015;  
77 Nottingham et al., 2015). These studies were conducted in forest ecosystems, mostly on P-  
78 limited tropical soils, short-term and biased towards carbon output, since changes in carbon input  
79 after fertilisation are more difficult to measure in forest systems and the assessment of net effects  
80 on SOC requires long-term observation. The response of SOC to P and K fertilisation of arable  
81 soils is not well documented, although these elements are considered to be the most important  
82 plant nutrients after nitrogen. As such, PK fertiliser application does usually have a positive  
83 effect on NPP, which should hypothetically counterbalance eventual losses via increased  
84 heterotrophic respiration. “P mining” at the cost of C, as the equivalent to “N mining” under P  
85 limited conditions has not been observed in soils (Craine et al., 2007). The Swedish long-term  
86 Soil Fertility Experiments were established between 1957 and 1966 (Carlgren and Mattsson,  
87 2001). These experiments are located across the country and cover a wide range of climatic and  
88 pedological conditions. They are unique because all of them have an almost identical

89 experimental design consists of a combination of different N and PK levels. Due to this meta-  
90 replication and the length of the experiments (>45 years) they have provided good opportunities  
91 to examine different hypotheses under *in situ* conditions throughout several decades (Simonsson  
92 et al., 2009; Williams et al., 2013). The effect of PK without N or N without PK, as examples for  
93 two extremely opposing nutrient regimes, has not been assessed. This comparison has the  
94 potential to provide powerful insights on long-term nutrient effects on SOC dynamics. The main  
95 question asked was: How did long-term N and PK fertilization in absence of the respective other  
96 influence SOC stocks and how did pedo-climatic factors affect those responses?

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## 98 2. Materials and Methods

### 99 2.1 Dataset

100 The dataset covered 10 sites from the ongoing Swedish long-term soil fertility experiments  
101 (Table 1), all located in central and southern Sweden (with five sites in each region). A total of  
102 16 combinations of mineral fertiliser applications (NPK), including four different N levels and  
103 four different PK levels, are being compared in two crop rotations (i.e. with or without manure  
104 application) with two replicates in a randomised split block design. The N levels (applied as  
105 ammonium nitrate before 1989 and as Nitro Chalk afterwards) are 0, 50, 100, and 200 kg for the  
106 southern Swedish sites and 0, 41, 82, 125 kg for central Sweden and will be referred to as 0N,  
107 1N, 2N and 3N in the text. The PK levels (P applied as mono superphosphate before 1994 and as  
108 triple superphosphate afterwards and K applied as potassium chloride) are 0 kg, replacement of  
109 the harvested PK, replacement plus 15 kg P and 40 kg K and replacement plus 30 kg P and 80 kg  
110 K for the southern Swedish sites, and 0 kg, replacement, replacement plus 20 kg P and 50 kg K

111 and replacement plus 30 kg P and 80 kg K for the central Swedish sites. These respective levels  
112 are referred to as 0PK, 1PK, 2PK and 3PK in the text. In this study, we analysed the effect on  
113 SOC of the four PK levels with no N fertiliser (Unfertilised control, 1PK<sub>0N</sub>, 2PK<sub>0N</sub>, 3PK<sub>0N</sub>) and  
114 the four N levels with no PK fertiliser (Unfertilised control, 1N<sub>0PK</sub>, 2N<sub>0PK</sub>, 3N<sub>0PK</sub>) in the rotation  
115 without farmyard manure. As a positive reference, we also assessed the 1NPK, 2NPK, 3NPK  
116 treatments. A total of 88 pairs of unfertilised vs. fertilised was investigated, with 3PK<sub>0N</sub> only  
117 being present in 8 experiments, while all other treatments were present in all 10 experiments.  
118 The four-year rotation investigated in the southern Swedish experiments consisted of barley,  
119 oilseed rape, winter wheat and sugar beet, while the six-year rotation investigated in central  
120 Sweden consisted of barley, oats, oilseed rape, winter wheat, oats and winter wheat. Initial soil  
121 characteristics and climate data are presented in Table 2. A detailed description of the soil profile  
122 and mineralogical composition of the soil at the sites is provided in numerous publications  
123 (Carlgren and Mattsson, 2001). A summary is provided in Table 2. The soils are sampled  
124 regularly to a depth of 20 cm (plough layer), air-dried and sieved to 2 mm. Initial plot-wise  
125 carbon data were available for all experiments. Samples with pH (H<sub>2</sub>O) exceeding 6.7 were  
126 treated with 2 M HCl to remove carbonates. Total carbon and nitrogen was determined by dry  
127 combustion using an elemental analyser (LECO-CNS-200, St. Joseph, MI, USA), P and K were  
128 both extracted using two different methods, according to Swedish standards: 0.1M NH<sub>4</sub>-lactate  
129 and 0.4M HOAc adjusted to pH 3.75 and subsequently analysed using an inductively coupled  
130 plasma apparatus (ICP-AES Optima 5300, Waltham, MA, USA). The initial carbon values were  
131 measured either with loss on ignition or the Walkley-Black wet oxidation, while there is no good  
132 technical documentation on that issue. However, due to the fact that we were investigating

133 differences between treatments, a possible bias induced by methodological considerations should  
134 be insignificant or very small.

## 135 2. 2 Calculation of carbon stocks and dynamics

136 The SOC stocks were calculated by multiplying soil bulk density, sampling depth and SOC  
137 concentration. To account for the fact that equal soil masses should be compared within one  
138 experiment (Ellert and Bettany, 1995), we used the initial average bulk density value for all  
139 treatments, which mathematically equals a depth adjustment to obtain the same soil mass from  
140 individual bulk density values (Poeplau et al., 2011). Bulk density values are reported for all  
141 experiments (Table 1) and did not change significantly over time (data not shown). The  
142 difference in SOC stocks between the unfertilised control and the respective treatment was then  
143 determined. The N and PK treatment effects on SOC stocks ( $\Delta\text{SOC}_{\text{treatment}}$ ) was calculated  
144 treatment-wise from the difference between initial ( $\Delta\text{SOC}_{\text{initial}}$ ) and final ( $\Delta\text{SOC}_{\text{final}}$ ) SOC stock  
145 in treatment and control plots:

$$146 \Delta\text{SOC}_{\text{treatment}} = \Delta\text{SOC}_{\text{final}} - \Delta\text{SOC}_{\text{initial}} \quad (\text{Eq. 1})$$

147 The southern Swedish experiments were initiated in 1957 but the experiment was first divided  
148 into subplots as they exist today in 1962, which was the first year in which plot-wise soil  
149 sampling was conducted. Thus for these five sites, 1962 was the reference year used to calculate  
150  $\Delta\text{SOC}_{\text{treatment}}$ . The difference in annual net flux of carbon ( $\Delta\text{Cflux}_{\text{net}}$ ) was calculated by dividing  
151  $\Delta\text{SOC}_{\text{treatment}}$  by the number of years between the first and the latest sampling. This represents the  
152 mean annual SOC stock change rate in a certain treatment compared with the unfertilised  
153 control. As a proxy for carbon inputs (Bolinder et al., 2007), we calculated relative differences in

154 annual yields between any considered fertilizer treatment and the unfertilised control, and  
155 averaged those differences over the whole period.

## 156 2. 3 Statistics

157 Linear mixed effect models were used to assess whether any of the fertiliser treatments had a  
158 significant effect on yields and SOC stock changes across sites. Treatment was used as the fixed  
159 effect, while site was used as a random effect. We calculated the model twice for both fertiliser  
160 regimes (N and PK fertilised soils), either using all levels as individual treatments or using levels  
161 1-3 together as one treatment (fertilised) testing it against the unfertilised control. Multiple linear  
162 regression models were used to explain the observed variability in the response to PK  
163 fertilisation with different explanatory variables for all three PK levels and all N levels  
164 separately. These were: clay content, sand content, average initial C, N,  $P_{\text{available}}$ ,  $K_{\text{available}}$   
165 contents, stoichiometric ratios of those elements, average initial soil pH, average losses of  
166 available phosphorus and potassium in the unfertilised control between the first and last  
167 sampling, final differences in available phosphorus and potassium between the unfertilised  
168 control and the fertilised treatment, final differences in soil pH between the control and the  
169 fertilised treatment and relative yield increase after fertilization. Due to the high number of  
170 potential explanatory variables, multi-model inference (R package MuMIn) was used for a-priori  
171 model selection and model averaging based on the Akaike Information Criterion (AIC). Error  
172 ranges shown in the text and in diagrams are standard deviations. All statistics were performed  
173 using the R software.

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## 175 3. Results



176 As expected, we observed an average increase in yields of 1% (1PK<sub>0N</sub>), 10% (2PK<sub>0N</sub>) and 15%  
177 (3PK<sub>0N</sub>) (Fig. 1A) following PK fertilisation. This increase was significant for 2PK<sub>0N</sub> (p=0.012),  
178 3PK<sub>0N</sub> (p=0.007) and for all pairs together (p=0.002). An equal, yet more pronounced response  
179 of NPP to fertilisation was observed for the different N levels (30 pairs) with significant  
180 increases of 45%, 80% and 86% for 1N<sub>0PK</sub>, 2N<sub>0PK</sub> and 3N<sub>0PK</sub> respectively.

181 Despite these expected positive effects on yields under both fertiliser regimes, we found  
182 contrasting responses of SOC stocks. In the PK fertilized soils, we detected a relative SOC stock  
183 depletion over time for 22 pairs out of 28 pairs (Figure 1B), which was statistically significant  
184 (p=0.004). All experiments have been running for more than 40 years and since we accounted for  
185 the initial differences in SOC between experimental plots, we considered that any influence of  
186 prior land use would be negligible. Only at one site, Fors, no decrease in SOC as a consequence  
187 of PK fertilisation was observed at all. On average, the negative effect of PK fertilisation  
188 increased with PK level: With 1PK<sub>0N</sub>, SOC stocks decreased by 2.0±4.3 Mg ha<sup>-1</sup> or 0.04±0.09  
189 Mg ha<sup>-1</sup>yr<sup>-1</sup> (ns); with 2PK<sub>0N</sub> they decreased by 2.3±5.8 Mg ha<sup>-1</sup> or 0.05±0.12 Mg ha<sup>-1</sup>yr<sup>-1</sup> (ns);  
190 and with 3PK<sub>0N</sub> they significantly (p=0.008) decreased by 4.2±3.3 Mg ha<sup>-1</sup> or 0.09±0.07 Mg ha<sup>-1</sup>  
191 yr<sup>-1</sup> (Fig. 2). SOC stocks under PK fertilisation alone did thus develop directly disproportional to  
192 yields, which is in contrast to the observations in the N fertilised soils (Fig. 2). Although SOC  
193 stock changes were not significant after long-term N fertilisation and even 1N<sub>0PK</sub> did lead to  
194 slight losses of SOC, the trend of increasing yields with increasing N level was tracked by the  
195 trend in SOC stocks (Fig. 2).

196 The effect of NPK fertilisation on NPP was synergistic, thus higher as the sum of the increases  
197 after PK and N fertilisation alone (Fig. 1 (a)). This strong increase in C input did also positively  
198 affect SOC stocks.

199 Observed annual SOC stock changes at different PK<sub>0N</sub> levels were partly explained by the  
200 variables considered, although the degree of explanation varied across PK levels. The changes in  
201 the 1PK<sub>0N</sub> treatments were best explained by mean annual temperature (MAT), average initial  
202 SOC concentration and average initial C:P<sub>available</sub> ratio, whereby all three variables were  
203 negatively correlated with annual SOC stock changes. The variables used explained 92% of the  
204 observed variation ( $R^2=0.92$ ) (Fig. 3). Losses of SOC were thus most pronounced at sites with  
205 high SOC concentration, a relatively warm climate and low available P. The effect of 2PK<sub>0N</sub>  
206 fertilisation on annual SOC stock changes was only significantly correlated with average initial  
207 soil pH (Fig. 3) ( $R^2=0.23$ ), while that of 0N 3PK was significantly correlated with mean annual  
208 precipitation (MAP) and initial soil carbon, achieving 70% explanation.

209 In contrast to the PK<sub>0N</sub> fertiliser levels, we found significant explanatory power of relative yield  
210 increases on SOC stock change in the 1N<sub>0PK</sub> levels, which indicates the link between carbon  
211 input and carbon stock dynamic. Furthermore, 1N<sub>0PK</sub> was negatively influenced by the C:P<sub>available</sub>  
212 ratio in the soil, with more negative changes in P-poor soils adding up to a total explanation of  
213 39% of the observed variance. C:P<sub>available</sub> ratio was also the only variable, which significantly  
214 explained some of the variability in observed SOC responses to 2N<sub>0PK</sub> ( $R^2=0.34$ , Fig. 3).

#### 215 4. Discussion

216 In most agricultural long-term experiments assessing the effects of mineral fertilisers on SOC,  
217 the focus is on the application rates or sources of nitrogen (Lugato et al., 2006; Malhi et al.,  
218 1997), because they might be more directly related to applied agronomy issues. The majority of  
219 existing studies did observe increased yields and SOC stocks with increased N or NPK  
220 fertilization, leading to the perception that carbon input is the major driver for SOC dynamics in

221 agricultural systems (Christopher and Lal, 2007). This pattern has been similarly observed in our  
222 study. Our finding that phosphorus and potassium (PK) in the absence of nitrogen fertilisation  
223 can have a negative net effect on SOC stocks, significantly so at the highest rate of PK  
224 fertilisation, has not been highlighted before. However, in several studies, P fertilisation was one  
225 among many other investigated fertiliser treatments without receiving much attention in the  
226 respective discussion. In those long-term experiments, mixed responses of P or PK fertilisation  
227 on SOC stocks have been observed. Some studies showed losses of SOC (Wyngaard et al., 2012;  
228 Yan et al., 2013; Zhu et al., 2007), while others found gains (Shao and Zheng, 2014; Zhengchao  
229 et al., 2013). The effect of P or PK fertilisation on yields was mostly slightly positive, which is in  
230 line with our findings for the Swedish sites. It is however a new observation that SOC stock  
231 changes do not always seem to follow changes in NPP and annual C inputs to soil, as the major  
232 source of SOC. This requires explanation. We considered four different mechanisms, each of  
233 which could explain parts of this observed depletion in SOC stocks under presumed increased  
234 carbon inputs:

235 1. *Fertilisation with P stimulates heterotrophic respiration.* A strong microbial response to  
236 P addition has been observed in several studies, mostly conducted in P-limited tropical  
237 forests (Cleveland and Townsend, 2006; Nottingham et al., 2015), but also in northern  
238 hardwood forests (Fisk et al. 2015). Craine et al. (2007) tested the effect of N and P  
239 addition on mineralisation for a wide range of different plant materials in different soils  
240 and found that C mineralisation decreased with N availability, while P fertilisation  
241 increased short- and long-term C mineralisation. They concluded that basic  
242 stoichiometric decomposition theory needs to be revised and carbon cycling models  
243 restructured to take into account the effect of P on SOC turnover. It is not entirely

244 understood, why N and P additions show opposing effects on microbial activity, but  
245 shifts in microbial community structure could play an important role (Ramirez et al.,  
246 2012). Recently, Hartman and Richardson (2013) found that the metabolic quotient of  
247 microbes (i.e., respiration per biomass) increased significantly following the addition of  
248 inorganic P, which could indicate that microbial activity in soil is largely controlled by P  
249 availability. In addition to microbial metabolism, the availability of P and N has also  
250 shown to trigger the resource allocation to exo-enzymes that are breaking down organic  
251 matter (Sinsabaugh and Moorhead, 1994). Extractable phosphorus has been a stronger  
252 predictor for C mineralization than land use (Strickland et al., 2010). Cleveland and  
253 Townsend (2006) reported an *in situ* increase in CO<sub>2</sub> efflux of 37% after P fertilisation  
254 and an *in vitro* 12% higher respiration in the P fertilised than in the unfertilised soil after  
255 adding glucose. Fisk et al. (2015) reported 28-122% higher CO<sub>2</sub> efflux in the P-fertilised  
256 soil than in the unfertilised soil, depending on added labile C substrates. Considering  
257 these observations, it is not an unrealistic hypothesis that an increase in respiration could  
258 be the most important mechanism underlying the observed changes in SOC stocks.  
259 Furthermore, the average relative phosphorus demand of microbial biomass corresponds  
260 to C:P ratio of 60:1 and a global average C:P ratio of 186:1 in soils is globally well  
261 constrained (Cleveland and Liptzin, 2007). In our study, the average C:P<sub>available</sub> ratio in  
262 the unfertilised control soils was 706:1. Thus, the stimulating effect of P on microbial  
263 activity might override the stimulating effect on plant growth, leading to net losses of  
264 SOC.

265 2. *Colonisation of roots with arbuscular mycorrhizal fungi (AMF) is reduced when easily*  
266 *available P is applied to soil* (Grant et al., 2005; Nagahashi et al., 1996). The main role of

267 AMF hyphae is to increase root uptake capacity for nutrients, including P, by enlarging  
268 the surface area of the root system (Pearson and Jakobsen, 1993). In return, the fungi  
269 receive carbon assimilates from the plant in the form of carbohydrates. It has been shown  
270 that plants can provide up to 17% of their photosynthetic carbon to the fungi (AMF,  
271 among other mycorrhiza types) (Hobbie and Hobbie, 2006). Clemmensen et al. (2013)  
272 identified root-associated fungi as important regulators of ecosystem carbon dynamics. In  
273 addition to the higher C input to soil through greater AMF colonisation, AMF-derived  
274 carbon (glomalin) may lead to the formation of more stable aggregates, protecting  
275 organic matter (Miller and Jastrow, 2000) and thereby preserving the SOC pool (Grant et  
276 al., 2005; Nagahashi et al., 1996; Rillig et al., 2001). Therefore, a decrease in AMF  
277 colonisation as a consequence of PK fertilisation can potentially have negative effects on  
278 SOC stocks.

279 3. *Fertilisation with P decreases plant root:shoot ratio.* A deficiency in major nutrients (N,  
280 P and K) generally leads to an increased root:shoot ratio (Wilson, 1988). Under N  
281 deficiency, a typical increase in root:shoot ratio is in the range of 15 to 50% (Hansson et  
282 al., 1987; Welbank et al., 1973). There is also evidence that P fertilisation can decrease  
283 the root:shoot ratio of crop plants (Ericsson, 1995; Marschner et al., 1996) compared with  
284 unfertilised soil. This change in root:shoot ratio occurs because nutrient deficiency forces  
285 crops to develop larger root systems than under conditions of sufficient nutrient supply  
286 (Marschner et al., 1996). Moreover, root-derived carbon has been found to contribute  
287 more to the stable soil carbon pool than shoot-derived carbon (Kätterer et al., 2011).  
288 Carbon inputs to the soil are usually yield-based estimates assuming stable carbon  
289 allocation coefficients to different plant parts across fertiliser regimes. This is common

290 practice in soil carbon modelling, although there are indications that it might be  
291 erroneous . The observed increase in yield for the 0N PK treatments which was 1, 10 and  
292 15% for 1PK<sub>0N</sub>, 2PK<sub>0N</sub> and 3PK<sub>0N</sub>, respectively lead to increased C input to the soil when  
293 assuming stable ratios of plant carbon allocation. However, relative to the unfertilised  
294 control, if a certain shift in root:shoot ratio did occur, PK fertilisation could actually have  
295 caused a decrease in total NPP. Thus, despite higher aboveground NPP, the smaller root  
296 inputs and the associated qualitative shift of the total carbon input might have  
297 significantly contributed to the observed SOC losses after PK<sub>0N</sub> fertilisation. However,  
298 the disproportional trend of yields and SOC stocks seems unrealistic to be explained by  
299 root:shoot ratio shift. Unchanged SOC stocks over all PK<sub>0N</sub> levels could be expected, but  
300 most likely not decreases.

301 4. *Stronger N mining leads to higher mineralisation of C.* In the 0N PK-fertilised plots with  
302 higher yields, more N is lost from the system through export by harvest. Such a negative  
303 effect of PK fertilisation on soil N has been observed by Glendining et al. (1997). This  
304 could have increased the N deficiency in the soil, leading to stronger “N mining” by  
305 microbes, a process in which nutrients are mobilised via decomposition of more stable  
306 organic matter (Fontaine et al., 2004). This might lead to loss of C from this pool and is  
307 in line with the findings of Kirkby et al. (2011) and (2013), who found that SOC  
308 sequestration relies on the availability of nutrients and that the stoichiometric ratio of  
309 C:N:P:S of stable soil organic matter is globally well constrained. We did not find any  
310 negative effect of PK fertilisation on SOC stocks in the presence of nitrogen fertilisation.  
311 This is in support of the hypothesis that the decreasing SOC stocks we observed were  
312 rather induced by a indirect response to PK fertilisation. However, the estimated surplus

313 of N extraction in the fertilised plots (with an average crop C:N ratio of 70) accounted for  
314 only 2-20% of the total soil N loss (average soil C:N ratio of 11) after 0N PK fertilisation.  
315 Consequently, this mechanism was probably only of minor importance in this study.  
316 Furthermore, Cleveland et al. (2006) found higher respiration when adding glucose with  
317 N+P as compared to the addition of glucose and N. This does indicate, that the  
318 stimulating effect of P on heterotrophic respiration is not restricted to N-deficient soils.

319 The relative importance of each of the first three mechanisms for SOC dynamics in the PK  
320 fertilised but N deficient regime remains speculative, since CO<sub>2</sub> efflux, abundance of AMF and  
321 belowground biomass production were not measured. However, the observed explanatory power  
322 of available C, available P, soil pH and the climate parameters MAT and MAP provide support  
323 for mechanisms (1) and (2), i.e. increased soil respiration and less AMF colonisation due to 0N  
324 PK fertilisation. Temperature, moisture, substrate availability and nutrient availability are key  
325 drivers for microbial activity. Surprisingly, SOC dynamics of the PK<sub>0N</sub> fertilised treatments were  
326 independent from relative yield increase and thus carbon input, while the variability in SOC  
327 dynamics in 1N<sub>0PK</sub> was partly explained by differences in relative yield increase. This fits the  
328 observation, that carbon dynamic in the PK<sub>0N</sub> treatments was decoupled from aboveground NPP,  
329 while it was positively correlated with aboveground NPP in the N<sub>0PK</sub> treatments. Furthermore,  
330 Clark et al. (1999) found twice the amount of AMF biomass in a soil with pH 4 compared with a  
331 soil of pH 5, which is in line with the more pronounced SOC losses we observed in low pH soils.  
332 Attributing the losses completely to changes in heterotrophic respiration, as developed above,  
333 appeared realistic when compared with the strong stimulations observed in other studies.  
334 However, the tendency of SOC losses after 1N 0PK fertilisation might support the hypothesis of  
335 significantly altered root:shoot ratio, which has been equally observed for N and P deficiencies

336 (Wilson, 1988). However, these losses could also be explicable by P mining due to a higher  
337 extraction of P in the N fertilised soils.

338 Craine et al. (2007) provided evidence for the N mining theory, but did not find any indications  
339 for P mining. This could potentially be related to the fact, that soil N is mainly stored in organic  
340 matter, while considerable fractions of soil P are also stored in anorganic forms, thus P mining  
341 would be less related to the break-down of organic matter. However, the fact that P availability  
342 did significantly trigger SOC responses to N fertilisation (Fig. 3, higher C:P<sub>available</sub> ratio led to  
343 less increase or more decrease in SOC) could denote that P mining did play a role in the studied  
344 experiments to some extent. The positive effect of N on NPP was much more pronounced than  
345 the effect of PK, which was most likely enough to overshadow any nutrient control on SOC  
346 decomposition. Differences between the two fertiliser regimes on carbon output or any of the  
347 above mentioned mechanisms is thus not possible to disclose in the present dataset. It can  
348 however be concluded, that SOC dynamics were input-driven under N fertilisation but P  
349 deficiency, but output-driven under PK fertilisation with N deficiency.

350 In this study, we were unable to separate the effect of P from a potential effect of K, since the  
351 two elements were applied in combination. Previously, Shao and Zheng (2014) found a slightly  
352 positive effect of K fertilisation on SOC stocks, whereas Yan et al. (2013) found no changes in  
353 yield and SOC stock compared with the unfertilised soil. Several studies have shown that K has  
354 an opposing, but less pronounced, effect on root:shoot ratio than P (Ericsson, 1995; Hackett,  
355 1968). Van Cleve and Moore (1978) found strongly increasing soil respiration with N and P  
356 fertilisation, but slightly decreasing soil respiration with K fertilisation. These studies indicate  
357 that the negative effect of PK<sub>0N</sub> fertilisation we observed in the Swedish experiments is most  
358 likely related to P fertilisation, while K could have even counterbalanced this effect to a certain



359 degree. A pH effect on SOC decomposition due to different fertiliser regimes (especially due to  
360 K fertilisation) could be excluded, since no systematic difference in pH was observed across  
361 treatments. This might be due to the fact that the soils were repeatedly limed, according to local  
362 agricultural practices. We cannot anticipate any other mechanism following K fertilisation in our  
363 study that could have led to SOC losses. However, it is recognized that the combined fertilisation  
364 of P and K used in this study does not allow a clear separation of the individual effects of these  
365 two elements. In the few available studies in which specifically P effects were studied, P was  
366 however also applied in combination with K as  $\text{KH}_2\text{PO}_4$  (Cleveland and Townsend, 2006; Craine  
367 et al., 2007).

## 368 5. Conclusions

369 For soils receiving no N fertiliser, a negative effect of phosphorus and potassium (PK)  
370 fertilisation on soil organic carbon (SOC) stocks was observed in nine out of the 10 meta-  
371 replicated Swedish long-term experiments studied. To our knowledge, it is the first study that  
372 shows a significant depletion of SOC stocks after PK fertilisation in the absence of N  
373 fertilisation. The processes involved might be diverse and are certainly not well studied, in  
374 contrast to the rising awareness of the importance of soil nutrients for net ecosystem carbon  
375 fluxes (Reed et al., 2011). According to the literature, of these two elements, P rather than K is  
376 likely to have the highest impact on ecosystem carbon fluxes. This finding requires attention,  
377 since a positive effect of N on NPP and SOC stock changes is widely accepted. It might be  
378 helpful to predict SOC stock changes after sudden stoichiometric imbalances in ecosystems as  
379 induced by fires, atmospheric deposition, or enhanced soil P extraction by legumes. Many  
380 studies focus on certain aspects of SOC cycling, such as soil respiration or the contribution of  
381 arbuscular mycorrhizal fungal (AMF) to the total SOC pool. The results obtained from these

382 studies are obviously helpful in identifying potential processes involved in soil responses, e.g. to  
383 certain management practices. However, as long as the relative contribution of each process  
384 remains unknown, it is difficult to refine our holistic knowledge on SOC dynamics. The  
385 observed negative effect of PK<sub>0N</sub> fertilisation, with its diverse potential causes, calls for a more  
386 integrated approach for studying SOC dynamics.

387

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538 Table 1: The 10 experimental sites used in this study with coordinates [ $^{\circ}$ N /  $^{\circ}$ E], starting year  
 539 (starting year in this study), year of last sampling, time span between first and last sampling  
 540 [years] and length of the rotation [years].

Site	Coordinates	Start of experiment	Last sampling	Time span	Rotation Length
Fjärdingslov	55 40 / 13 23	1957 (1962)	2011	49	4
Orup	55 82 / 13 50	1957 (1962)	2011	49	4
Örja	55 88 / 12 87	1957 (1962)	2011	49	4
S. Ugglarp	55 63 / 13 43	1957 (1962)	2007	45	4
Ekebo	55 98 / 12 87	1957 (1962)	2011	49	4
Vreta Kloster	58 50 / 15 50	1966	2007	45	6
Högåsa	58 50 / 15 45	1966	2007	45	6
Bjertorp	58 23 / 13 13	1966	2007	45	6
Kungsängen	59 83 / 17 67	1963	2011	49	6
Fors	60 33 / 17 48	1963	2011	49	6

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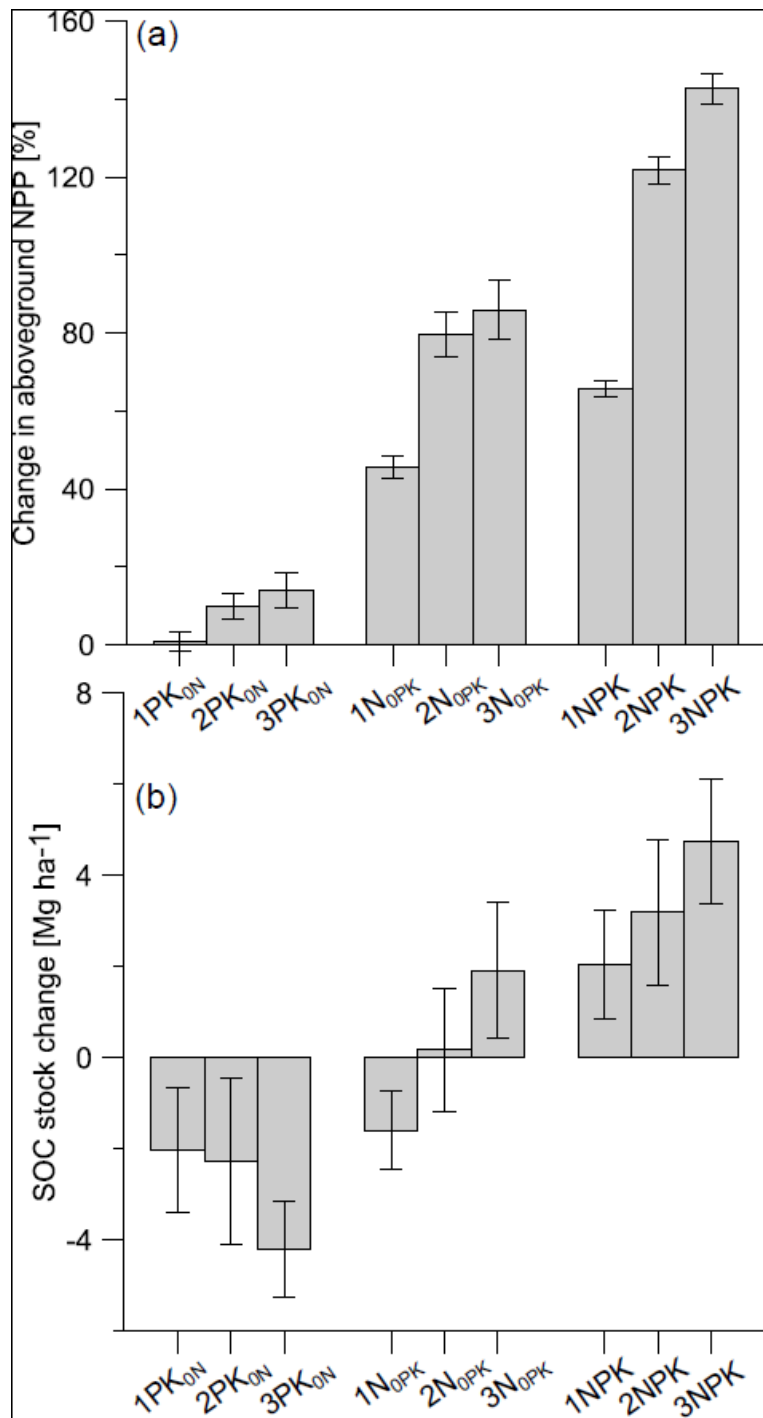
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547 Table 2: Basic soil parameters and climate data for the 10 study sites at the beginning of the  
 548 experiments: clay and sand content [%], soil organic carbon content [%], soil pH, bulk density [g  
 549 cm<sup>-3</sup>], mean annual temperature (MAT) [°C] and mean annual precipitation (MAP) [mm].

Site	Clay	Sand	SOC	pH	Bulk density	MAT	MAP
Fjärdingslov	14	62	1.4	7.9	1.66	7.7	550
Orup	12	59	2.4	6.6	1.51	8.3	769
Örja	23	52	1.1	7.8	1.72	8.3	593
S. Ugglarp	12	63	1.5	6.7	1.50	7.7	686
Ekebo	18	47	3.1	6.9	1.44	8.2	622
Vreta Kloster	48	8	2.1	6.2	1.43	6.4	527
Högåsa	7	78	2.4	6.7	1.38	6.4	527
Bjertorp	30	16	2.2	6	1.37	6.5	593
Kungsängen	56	4	2.1	7.1	1.31	6	543
Fors	18	24	2.2	7.7	1.49	5.5	613

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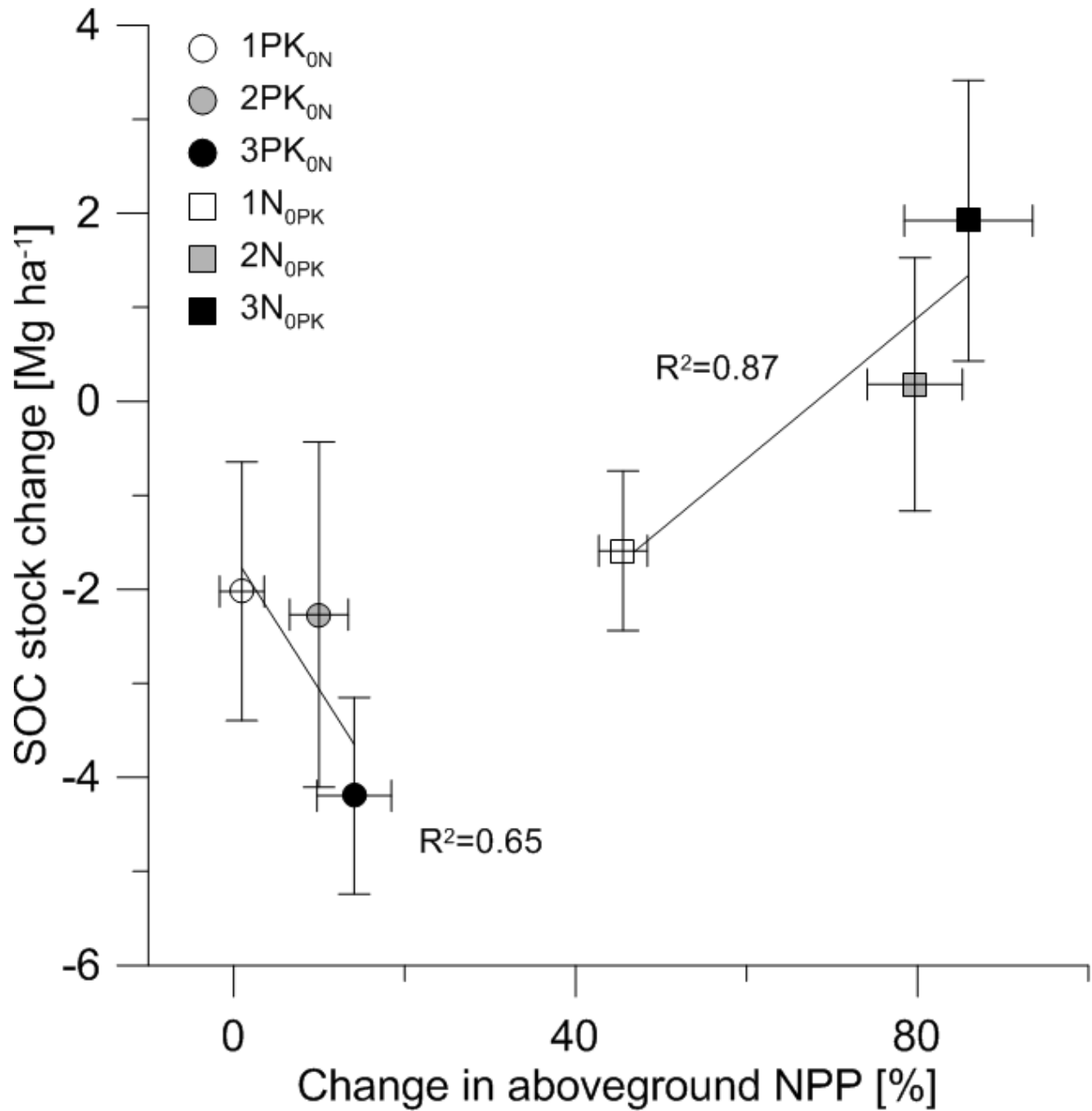
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553 Figure 1: a) Average relative yield differences and b) Average soil organic carbon (SOC) stock  
 554 differences between fertilised and unfertilised soils for all investigated levels of fertilisation  
 555 (PK<sub>ON</sub>, N<sub>OPK</sub>, NPK) with standard errors.

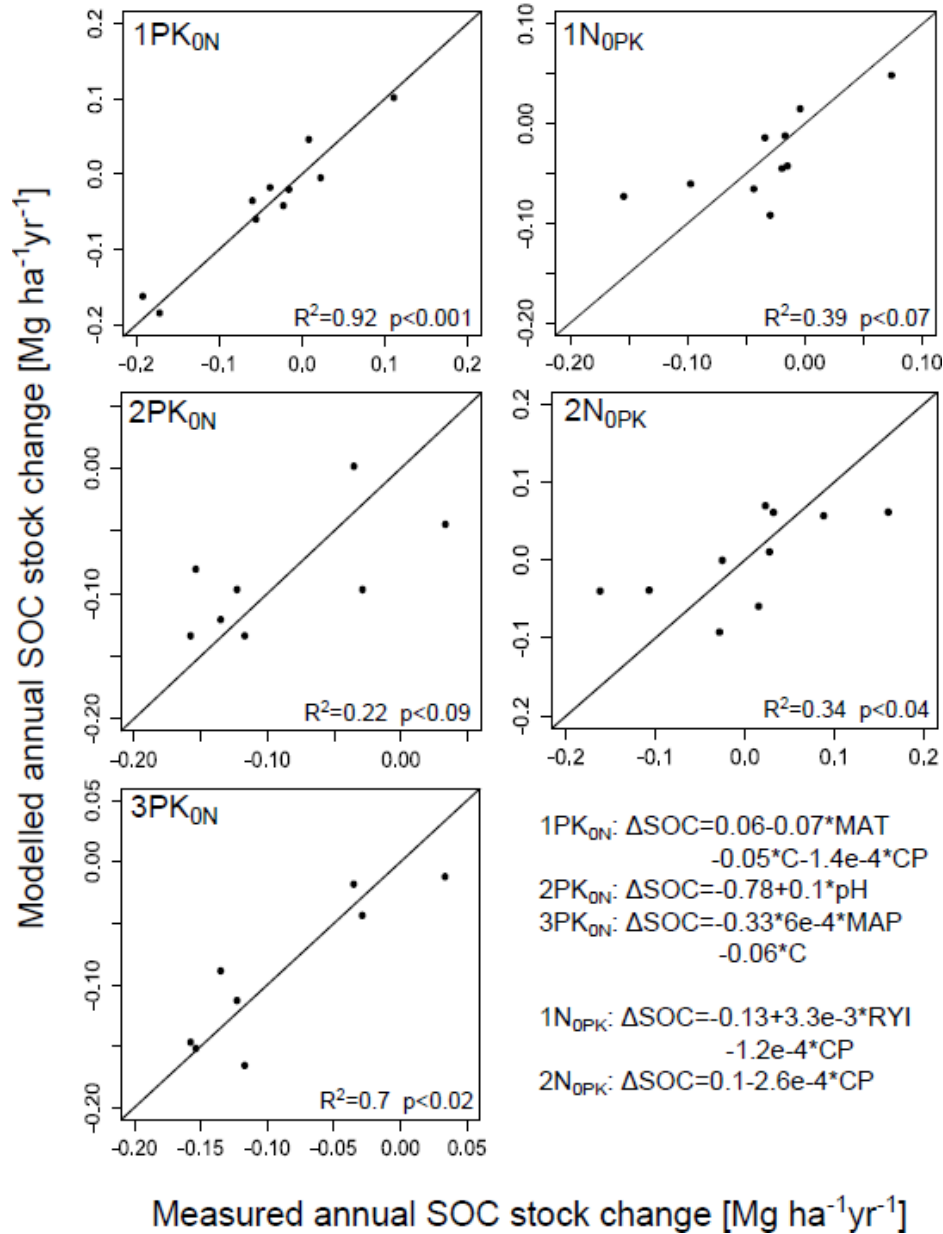




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557 Figure 2: Correlation of average SOC stock differences between fertilised and unfertilised soils  
 558 and average yield differences in fertilised and unfertilised soils with regression lines for the P  
 559 and N fertilised regimes separately. Error bars indicate standard errors.

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Figure 3: Comparison of measured and modelled annual soil organic carbon (SOC) stock change [Mg ha<sup>-1</sup>yr<sup>-1</sup>] for all phosphorus-potassium (PK) levels and two N levels, with model equations in which MAT is mean annual temperature [°C], MAP mean annual precipitation [mm], pH average initial soil pH, C average initial SOC concentration [%], CP the average initial C:P<sub>available</sub> ratio and RYI the relative yield increase [%].