Response to Referee #1:

1. We agree, deleted the sentence and replaced it by the following: “How did long-term N and PK fertilization in absence of the respective other influence SOC stocks and how did pedo-climatic factors affect those responses?”

2. We agree that the sample preparation was not described in sufficient detail. A) soils were air-dried and sieved to 2 mm. B) Unfortunately, there is no good technical documentation on that issue. However, we know that two of the earlier methods (LOI and Walkley-Black) have been used in the past for SOC analysis. In our case, we are looking at relative differences between treatments. Therefore, the possible bias induced by methodological considerations should be insignificant or very small. C) Samples with pH (H_2O) exceeding 6.7 were pre-treated with 2 M HCl to remove carbonates. We complemented the M+M section accordingly.

3. We have now added the 1NPK, 2NPK and 3NPK treatments as a “positive reference” for the reduced fertilisation treatments. However, the focus of this manuscript should remain the comparison of only PK vs. only N. Therefore we have only added those treatments in Figure 1 and present the results in the results section shortly as follows: “The effect of NPK fertilisation on NPP was synergistic, thus higher as the sum of the increases after PK and N fertilisation alone (Fig. 1 (a)). This strong increase in C input did also positively affect SOC stocks.”

4. We agree that the discussion was relatively weak regarding the explanatory variables. But due to the inconsistency in the models (different explanatory variables), the discussion of these models is not straightforward. However, we now added the following sentences: “Surprisingly, SOC dynamics of the PK_{0N} fertilised treatments were independent from relative yield increase and thus carbon input, while the variability in SOC dynamics in $1N_{0PK}$ was partly explained by differences in relative yield increase. This fits the observation, that carbon dynamic in the PK_{0N} treatments was decoupled from aboveground NPP, while it was positively correlated with aboveground NPP in the N_{0PK} treatments.”

5. The presentation of Figure 2 was chosen to better visualise that the SOC/yield relation is completely opposing between the two fertilisation treatments. As such, the graph carries a clear message. High regression coefficients (although only for 3 points) is “nice to have”, but not extremely important for the message. We have now added error bars in both directions.

6. We did not understand the suggestion with the datapoint size, since all datapoints represent a combination of the explanatory variables used. We however understand the criticism that the figure might not be of much value and that the statistical results could also be presented in a table. It might be a matter of taste, but we think that visualising the model fits, although it might be somewhat abstract, helps the reader to directly get an idea of how good the used variables could explain the data and how the different experiments distribute around the 1:1 line. I myself prefer graphs, and the manuscript is not overloaded with graphs (only 2 others). Therefore we do not think that it is absolutely necessary to change it into a table.

Specific comments:

1. We agree and added a reference

2. The reference was missing. We now added it to the reference list.

3. We changed this accordingly.

4. We changed this accordingly.

5. We changed this into SOC stock changes.

6. We agree that this sentence belongs to M+M and also added the N and NPK treatments, which have not been counted in this sentence (which was from an earlier manuscript version). The sentence now reads: “A total of 88 pairs of unfertilised vs. fertilised was investigated, with $3PK_{0N}$ only being present in 8 experiments, while all other treatments were present in all 10 experiments.” We also had to change the next sentence, which now reads: “As expected, we observed an average increase in yields of 1% ($1PK_{0N}$), 10% ($2PK_{0N}$) and 15% ($3PK_{0N}$) (Fig. 1A) following PK fertilisation.”

7. We changed that accordingly.

Response to Referee #2:

We agree that increases in P availability can also increase SOC decomposition in N-rich soils. One indication for that might be the results of Clevleand et al. (2006), who found higher respiration when N+P was added as compared to N alone. We therefore added the following sentence at the end of the hypothesis 4 section: “Furthermore, Cleveland et al. (2006) found higher respiration when adding glucose with N+P as compared to the addition of glucose and N. This does indicate, that the stimulating effect of P on heterotrophic respiration is not restricted to N–poor soils.”.

The experimental design of this study does however hamper such an investigation. We have checked SOC stock changes with increasing PK fertilization also with N (and different N levels) as a reference (instead of taking the unfertilized treatment as a reference). However, the combination of N+PK did always strongly increase net primary production and thus carbon inputs, which did most likely override the effects of PK on decomposition (because we find higher stocks under NPK than under N). Therefore we cannot confirm this hypothesis with the data we have. We only found this net negative effect of PK fertilization when compared to the unfertilized controls.

We agree that the paper was lacking some thoughts about the implications of our findings. We therefore added the following sentence to the conclusions: “It (the finding) might be helpful to model SOC stock changes after sudden stoichiometric imbalances in ecosystems as induced by fires, atmospheric deposition, or enhanced soil P extraction by legumes.” We do not think that it makes much sense to develop even more on this aspect in the discussion because that issue lies beyond the scope of the manuscript.

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

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Abstract

Increasing soil organic carbon (SOC) in agricultural soils can mitigate atmospheric CO_2 concentration and also contribute to increase soil fertility and ecosystem resilience. The role of major nutrients on SOC dynamics is complex, due to simultaneous effects on net primary productivity (NPP) that influence crop residue carbon inputs and on the rate of heterotrophic respiration (carbon outputs). This study investigated the effect on SOC stocks of three different levels of phosphorus and potassium (PK) fertilisation rates in the absence of nitrogen fertilisation and of three different levels of nitrogen in the absence of PK. This was done by analysing data from 10 meta-replicated Swedish long-term field experiments (>45 years). With N fertilisation, SOC stocks followed yield increases. However, for all PK levels, we found average SOC losses ranging from -0.04±0.09 Mg ha^{-1} yr^{-1} (ns) for the lowest to -0.09±0.07 Mg $ha^{-1}yr^{-1}$ ($p=0.008$) for the highest application rate, while crop yields as a proxy for carbon input increased significantly with PK fertilization by 1, 10 and 15%. We conclude that SOC dynamics are mainly output-driven in the PK fertilised regime but mostly input-driven in the N fertilised regime, due to the much more pronounced response of NPP to N than to PK fertilisation. It has been established that P rather than K is the element affecting ecosystem carbon fluxes, where P fertilisation has been shown to: i) stimulate heterotrophic respiration, ii) reduce the abundance of arbuscular mycorrhizal fungi and iii) decrease crop root:shoot ratio, leading to higher root-derived carbon input. The higher export of N in the PK fertilised plots in this study could iv) have led to increased N mining and thus mineralisation of organic matter. More integrated experiments are needed to gain a better understanding of the relative importance of each of the above-mentioned mechanisms leading to SOC losses after P addition.

1. Introduction

Maintaining or increasing soil organic carbon (SOC) stocks in agricultural soil is important for ecosystem resilience, soil fertility and sustainable crop production. Sequestration of SOC is also an important climate mitigation option (~~Lugato et al., 2006~~Lal, 2003~~)~~; Lugato et al., 2006). The annual export of plant biomass demands a sufficient nutrient supply, mainly of nitrogen (N), phosphorus (P) and potassium (K). To cope with steadily increasing global food demand and limitations to spatial expansion of agricultural area, the optimal balance between production and sustaining soil fertility has to be found. The magnitude and dynamics of SOC stocks are determined by the ratio between inputs and outputs of carbon. It is a well-established fact that mineral (NPK) fertiliser has a strong positive effect on net primary production (NPP) and thus on carbon inputs (Kätterer et al. 2012). In three global meta-analyses, the increase in topsoil SOC following mineral nitrogen fertilisation was reported to range from 3.5 to 8% (Alvarez, 2005; Ladha et al., 2011; Lu et al., 2011). Mineral fertilisation is therefore generally recommended as a suitable management practice for SOC sequestration (Lugato et al., 2006). In many long-term experiments with differing fertiliser treatments, SOC stocks have been well explained by measured or estimated NPP ~~(e.g. Kätterer et al. 2013).~~(Kätterer et al., 2012). However, the effect of N fertilisation on the corresponding C output by heterotrophic respiration and the mechanisms involved are still not fully understood. Nitrogen fertilisation has been shown to decrease respiratory C losses, especially in combination with a labile C source amendment. This is mostly explained by the “N mining”-theory, which predicts accelerated microbial decomposition of more recalcitrant, energy-poor organic matter in the search of nitrogen under N deficiency. Energy for this break-down is thereby derived from labile C, which in turn cannot efficiently be used for biosynthesis (Bradford et al., 2008; Craine et al., 2007; Milcu et al., 2011; Schimel and

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Weintraub, 2003). Cleveland and Liptzin (2007) reported a globally well constrained microbial biomass C:N:P ratio of 60:7:1, which indicates stoichiometric ~~constrains~~constraints for microbial growth. Recently, Murphy et al. (2015) observed that specific N-rich compounds of soil organic matter were primed after glucose addition. This observation can be interpreted as selective N-mining. The opposite effect, i.e. acceleration of C mineralisation by N addition, has also been reported (Allen and Schlesinger, 2004; Milcu et al., 2011; Reed et al., 2011), indicating that N can be a limiting factor for microbial breakdown. To date, studies about the effect of mineral fertiliser on SOC have mainly focused on N or combined N, P and K fertiliser, because nitrogen has the most obvious effects on the carbon cycle and NPK is the most common fertiliser combination used in commercial agriculture. However, it has recently been shown that P or PK fertilisation can have stimulating effects on heterotrophic respiration (Cleveland and Townsend, 2006; Cleveland et al., 2002; Fisk et al., 2015; Nottingham et al., 2015). These studies were conducted in forest ecosystems, mostly on P-limited tropical soils, short-term and biased towards carbon output, since changes in carbon input after fertilisation are more difficult to measure in forest systems and the assessment of net effects on SOC requires long-term observation. The response of SOC to P and K fertilisation of arable soils is not well documented, although these elements are considered to be the most important plant nutrients after nitrogen. As such, PK fertiliser application does usually have a positive effect on NPP, which should hypothetically ~~counterbalances~~counterbalance eventual losses via increased heterotrophic respiration. “P mining” at the cost of C, as the equivalent to “N mining” under P limited conditions has not been observed in soils (Craine et al., 2007). The Swedish long-term Soil Fertility Experiments were established between 1957 and 1966 (Carlgren and Mattsson, 2001). These experiments are located across the country and cover a wide range of climatic and pedological conditions. They

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are unique because all of them have an almost identical experimental design consists of a combination of different N and PK levels. Due to this meta-replication and the length of the experiments (>45 years) they have provided good opportunities to examine different hypotheses under *in situ* conditions throughout several decades (Simonsson et al., 2009; Williams et al., 2013). The effect of PK without N or N without PK, as examples for two extremely opposing nutrient regimes, has not been assessed. This comparison has the potential to provide powerful insights on long-term nutrient effects on SOC dynamics. The main question asked was: ~~Do~~How did long-term N and PK ~~fertilizer applications~~fertilization in absence of the respective other ~~deviate in their effects on~~influence SOC stocks ~~regarding magnitude~~ and ~~direction?~~how did pedo-climatic factors affect those responses?

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2. Materials and Methods

2.1 Dataset

The dataset covered 10 sites from the ongoing Swedish long-term soil fertility experiments (Table 1), all located in central and southern Sweden (with five sites in each region). A total of 16 combinations of mineral fertiliser applications (NPK), including four different N levels and four different PK levels, are being compared in two crop rotations (i.e. with or without manure application) with two replicates in a randomised split block design. The N levels (applied as ammonium nitrate before 1989 and as Nitro Chalk afterwards) are 0, 50, 100, and 200 kg for the southern Swedish sites and 0, 41, 82, 125 kg for central Sweden and will be referred to as 0N, 1N, 2N and 3N in the text. The PK levels (P applied as mono superphosphate before 1994 and as triple superphosphate afterwards and K applied as potassium chloride) are 0 kg, replacement of the harvested PK, replacement plus 15 kg P and 40 kg K and replacement plus 30 kg P and 80 kg K for the southern Swedish sites, and 0 kg, replacement, replacement plus 20 kg P and 50 kg K and replacement plus 30 kg P and 80 kg K for the central Swedish sites. These respective levels are referred to as 0PK, 1PK, 2PK and 3PK in the text. In this study, we analysed the effect on SOC of the four PK levels with no N fertiliser (Unfertilised control, 1PK$_{0N}$, 2PK$_{0N}$, 3PK$_{0N}$) and the four N levels with no PK fertiliser (Unfertilised control, 1N$_{0PK}$, 2N$_{0PK}$, 3N$_{0PK}$) in the rotation without farmyard manure. As a positive reference, we also assessed the 1NPK, 2NPK, 3NPK treatments. A total of 88 pairs of unfertilised vs. fertilised was investigated, with 3PK$_{0N}$ only being present in 8 experiments, while all other treatments were present in all 10 experiments. The four-year rotation investigated in the southern Swedish experiments consisted of barley, oilseed rape, winter wheat and sugar beet, while the six-year rotation investigated in central Sweden consisted of barley, oats, oilseed rape, winter wheat, oats and winter wheat. Initial soil characteristics and climate data are presented in Table 2. A detailed description of the soil profile and mineralogical composition of the soil at the sites is provided in numerous publications (Carlgren and Mattsson, 2001). A summary is provided in Table 2. The soils are sampled regularly to a depth of 20 cm (plough layer~~)~~), air-dried and ~~initial~~sieved to 2 mm. Initial plot-wise carbon data ~~are~~were available for all experiments. Samples with pH (H_2O) exceeding 6.7 were treated with 2 M HCl to remove carbonates. Total carbon and nitrogen was determined by dry combustion using an elemental analyser (LECO-CNS-200, St. Joseph, MI, USA), P and K were both extracted using two different methods, according to Swedish standards: 0.1M NH_4-lactate and 0.4M HOAc adjusted to pH 3.75 and subsequently analysed using an inductively coupled plasma apparatus (ICP-AES Optima 5300, Waltham, MA, USA). The initial carbon values were measured either with loss on ignition or the Walkley-Black wet oxidation, while there is no good technical documentation on that issue. However, due to the fact that we were investigating differences between treatments, a possible bias induced by methodological considerations should be insignificant or very small.

2. 2 Calculation of carbon stocks and dynamics

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

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$$\Delta SOC_{treatment} = \Delta SOC_{final} - \Delta SOC_{initial} \qquad \text{(Eq. 1)}$$

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

2. 3 Statistics

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

3. Results

~~A total of 28 pairs of unfertilised and PK fertilised treatment were investigated, with 1 PK and 2 PK being present at all 10 sites and 3 PK only at 8 sites.~~ As expected, we observed an average increase in yields of 1% (1PK$_{0N}$), 10% (2PK$_{0N}$) and 15% (3PK$_{0N}$) (Fig. 1A~~).~~) following PK fertilisation. This increase was significant for 2PK$_{0N}$ (p=0.012), 3PK$_{0N}$ (p=0.007) and for all pairs together (p=0.002). An equal, yet more pronounced response of NPP to fertilisation was observed for the different N levels (30 pairs) with significant increases of 45%, 80% and 86% for 1N$_{0PK}$, 2N$_{0PK}$ and 3N$_{0PK}$ respectively.

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

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

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

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

4. Discussion

In most agricultural long-term experiments assessing the effects of mineral fertilisers on SOC, the focus is on the application rates or sources of nitrogen (Lugato et al., 2006; Malhi et al.,

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1997), because they might be more directly related to applied agronomy issues. The majority of existing studies did observe increased yields and SOC stocks with increased N or NPK fertilization, leading to the perception that carbon input is the major driver for SOC dynamics in agricultural systems (Christopher and Lal, 2007). This pattern has been similarly observed in our study. Our finding that phosphorus and potassium (PK) in the absence of nitrogen fertilisation can have a negative net effect on SOC stocks, significantly so at the highest rate of PK fertilisation, has not been highlighted before. However, in several studies, P fertilisation was one among many other investigated fertiliser treatments without receiving much attention in the respective discussion. In those long-term experiments, mixed responses of P or PK fertilisation on SOC stocks have been observed. Some studies showed losses of SOC (Wyngaard et al., 2012; Yan et al., 2013; Zhu et al., 2007), while others found gains (Shao and Zheng, 2014; Zhengchao et al., 2013). The effect of P or PK fertilisation on yields was mostly slightly positive, which is in line with our findings for the Swedish sites. It is however a new observation that SOC stock changes do not always seem to follow changes in NPP and annual C inputs to soil, as the major source of SOC. This requires explanation. We considered four different mechanisms, each of which could explain parts of this observed depletion in SOC stocks under presumed increased carbon inputs:

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1. *Fertilisation with P stimulates heterotrophic respiration*. A strong microbial response to P addition has been observed in several studies, mostly conducted in P-limited tropical forests (Cleveland and Townsend, 2006; Nottingham et al., 2015), but also in northern hardwood forests (Fisk et al. 2015). Craine et al. (2007) tested the effect of N and P addition on mineralisation for a wide range of different plant materials in different soils and found that C mineralisation decreased with N availability, while P fertilisation

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increased short- and long-term C mineralisation. They concluded that basic stoichiometric decomposition theory needs to be revised and carbon cycling models restructured to take into account the effect of P on SOC turnover. It is not entirely understood, why N and P additions show opposing effects on microbial activity, but shifts in microbial community structure could play an important role (Ramirez et al., 2012). Recently, Hartman and Richardson (2013) found that the metabolic quotient of microbes (i.e., respiration per biomass) increased significantly following the addition of inorganic P, which could indicate that microbial activity in soil is largely controlled by P availability. In addition to microbial metabolism, the availability of P an N has also shown to trigger the resource allocation to exo-enzymes that are breaking down organic matter (Sinsabaugh and Moorhead, 1994). Extractable phosphorus has been a stronger predictor for C mineralization than land use (Strickland et al., 2010). Cleveland and Townsend (2006) reported an *in situ* increase in CO_2 efflux of 37% after P fertilisation and an *in vitro* 12% higher respiration in the P fertilised than in the unfertilised soil after adding glucose. Fisk et al. (2015) reported 28-122% higher CO_2 efflux in the P-fertilised soil than in the unfertilised soil, depending on added labile C substrates. Considering these observations, it is not an unrealistic hypothesis that an increase in respiration could be the most important mechanism underlying the observed changes in SOC stocks. Furthermore, the average relative phosphorus demand of microbial biomass corresponds to C:P ratio of 60:1 and a global average C:P ratio of 186:1 in soils is globally well constrained (Cleveland and Liptzin, 2007). In our study, the average C:$P_{available}$ ratio in the unfertilised control soils was 706:1. Thus, the stimulating effect of P on microbial

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activity might override the stimulating effect on plant growth, leading to net losses of SOC.

2. *Colonisation of roots with arbuscular mycorrhizal fungi (AMF) is reduced when easily available P is applied to soil* (Grant et al., 2005; Nagahashi et al., 1996). The main role of AMF hyphae is to increase root uptake capacity for nutrients, including P, by enlarging the surface area of the root system (Pearson and Jakobsen, 1993). In return, the fungi receive carbon assimilates from the plant in the form of carbohydrates. It has been shown that plants can provide up to 17% of their photosynthetic carbon to the fungi (AMF, among other mycorrhiza types) (Hobbie and Hobbie, 2006). Clemmensen et al. (2013) identified root-associated fungi as important regulators of ecosystem carbon dynamics. In addition to the higher C input to soil through greater AMF colonisation, AMF-derived carbon (glomalin) may lead to the formation of more stable aggregates, protecting organic matter (Miller and Jastrow, 2000) and thereby preserving the SOC pool (Grant et al., 2005; Nagahashi et al., 1996; Rillig et al., 2001). Therefore, a decrease in AMF colonisation as a consequence of PK fertilisation can potentially have negative effects on SOC stocks.
3. *Fertilisation with P decreases plant root:shoot ratio*. A deficiency in major nutrients (N, P and K) generally leads to an increased root:shoot ratio (Wilson, 1988). Under N deficiency, a typical increase in root:shoot ratio is in the range of 15 to 50% (Hansson et al., 1987; Welbank et al., 1973). There is also evidence that P fertilisation can decrease the root:shoot ratio of crop plants (Ericsson, 1995; Marschner et al., 1996) compared with unfertilised soil. This change in root:shoot ratio occurs because nutrient deficiency forces crops to develop larger root systems than under conditions of sufficient nutrient supply

(Marschner et al., 1996). Moreover, root-derived carbon has been found to contribute more to the stable soil carbon pool than shoot-derived carbon (Kätterer et al., 2011). Carbon inputs to the soil are usually yield-based estimates assuming stable carbon allocation coefficients to different plant parts across fertiliser regimes. This is common practice in soil carbon modelling, although there are indications that it might be erroneous . The observed increase in yield for the 0N PK treatments which was 1, 10 and 15% for $1PK_{0N}$, $2PK_{0N}$ and $3PK_{0N}$, respectively lead to increased C input to the soil when assuming stable ratios of plant carbon allocation. However, relative to the unfertilised control, if a certain shift in root:shoot ratio did occur, PK fertilisation could actually have caused a decrease in total NPP. Thus, despite higher aboveground NPP, the smaller root inputs and the associated qualitative shift of the total carbon input might have significantly contributed to the observed SOC losses after PK_{0N} fertilisation. However, the disproportional trend of yields and SOC stocks seams unrealistic to be explained by root:shoot ratio shift. Unchanged SOC stocks over all PK_{0N} levels could be expected, but most likely not decreases.

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4. *Stronger N mining leads to higher mineralisation of C*. In the 0N PK-fertilised plots with higher yields, more N is lost from the system through export by harvest. Such a negative effect of PK fertilisation on soil N has been observed by Glendining et al. (1997). This could have increased the N deficiency in the soil, leading to stronger “N mining” by microbes, a process in which nutrients are mobilised via decomposition of more stable organic matter (Fontaine et al., 2004). This might lead to loss of C from this pool and is in line with the findings of Kirkby et al. (2011) and (2013), who found that SOC sequestration relies on the availability of nutrients and that the stoichiometric ratio of

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C:N:P:S of stable soil organic matter is globally well constrained. We did not find any negative effect of PK fertilisation on SOC stocks in the presence of nitrogen fertilisation ~~(data not shown).~~. This is in support of the hypothesis that the decreasing SOC stocks we observed were rather induced by a indirect response to PK fertilisation. However, the estimated surplus of N extraction in the fertilised plots (with an average crop C:N ratio of 70) accounted for only 2-20% of the total soil N loss (average soil C:N ratio of 11) after 0N PK fertilisation. Consequently, this mechanism was probably only of minor importance in this study. Furthermore, Cleveland et al. (2006) found higher respiration when adding glucose with N+P as compared to the addition of glucose and N. This does indicate, that the stimulating effect of P on heterotrophic respiration is not restricted to N–deficient soils.

The relative importance of each of the first three mechanisms for SOC dynamics in the PK fertilised but N deficient regime remains speculative, since CO_2 efflux, abundance of AMF and belowground biomass production were not measured. However, the observed explanatory power of available C, available P, soil pH and the climate parameters MAT and MAP provide support for mechanisms (1) and (2), i.e. increased soil respiration and less AMF colonisation due to 0N PK fertilisation. Temperature, moisture, substrate availability and nutrient availability are key drivers for microbial activity. Surprisingly, SOC dynamics of the PK_{0N} fertilised treatments were independent from relative yield increase and thus carbon input, while the variability in SOC dynamics in $1N_{0PK}$ was partly explained by differences in relative yield increase. This fits the observation, that carbon dynamic in the PK_{0N} treatments was decoupled from aboveground NPP, while it was positively correlated with aboveground NPP in the N_{0PK} treatments. Furthermore, Clark et al. (1999) found twice the amount of AMF biomass in a soil with pH 4 compared with a soil of pH 5, which is in line with the more pronounced SOC losses we observed in low pH soils. Attributing the losses completely to changes in heterotrophic respiration, as developed above, appeared realistic when compared with the strong stimulations observed in other studies. However, the tendency of SOC losses after 1N 0PK fertilisation might support the hypothesis of significantly altered root:shoot ratio, which has been equally observed for N and P deficiencies (Wilson, 1988). However, these losses could also be explicable by P mining due to a higher extraction of P in the N fertilised soils.

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

In this study, we were unable to separate the effect of P from a potential effect of K, since the two elements were applied in combination. Previously, Shao and Zheng (2014) found a slightly positive effect of K fertilisation on SOC stocks, whereas Yan et al. (2013) found no changes in yield and SOC stock compared with the unfertilised soil. Several studies have shown that K has an opposing, but less pronounced, effect on root:shoot ratio than P (Ericsson, 1995; Hackett, 1968). Van Cleve and Moore (1978) found strongly increasing soil respiration with N and P fertilisation, but slightly decreasing soil respiration with K fertilisation. These studies indicate that the negative effect of PK_{0N} fertilisation we observed in the Swedish experiments is most likely related to P fertilisation, while K could have even counterbalanced this effect to a certain degree. A pH effect on SOC decomposition due to different fertiliser regimes (especially due to K fertilisation) could be excluded, since no systematic difference in pH was observed across treatments. This might be due to the fact that the soils were repeatedly limed, according to local agricultural practices. We cannot anticipate any other mechanism following K fertilisation in our study that could have led to SOC losses. However, it is recognized that the combined fertilisation of P and K used in this study does not allow a clear separation of the individual effects of these two elements. In the few available studies in which specifically P effects were studied, P was however also applied in combination with K as KH_2PO_4 (Cleveland and Townsend, 2006; Craine et al., 2007).

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5. Conclusions

For soils receiving no N fertiliser, a negative effect of phosphorus and potassium (PK) fertilisation on soil organic carbon (SOC) stocks was observed in nine out of the 10 meta-replicated Swedish long-term experiments studied. To our knowledge, it is the first study that shows a significant depletion of SOC stocks after PK fertilisation in the absence of N fertilisation. The processes involved might be diverse and are certainly not well studied, in contrast to the rising awareness of the importance of soil nutrients for net ecosystem carbon fluxes (Reed et al., 2011). According to the literature, of these two elements, P rather than K is likely to have the highest impact on ecosystem carbon fluxes. ~~These findings require~~This finding

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requires attention, since a positive effect of N on NPP and SOC stock changes is widely accepted. It might be helpful to predict SOC stock changes after sudden stoichiometric imbalances in ecosystems as induced by fires, atmospheric deposition, or enhanced soil P extraction by legumes. Many studies focus on certain aspects of SOC cycling, such as soil respiration or the contribution of arbuscular mycorrhizal fungal (AMF) to the total SOC pool. The results obtained from these studies are obviously helpful in identifying potential processes involved in soil responses, e.g. to certain management practices. However, as long as the relative contribution of each process remains unknown, it is difficult to refine our holistic knowledge on SOC dynamics. The observed negative effect of PK_{0N} fertilisation, with its diverse potential causes, calls for a more integrated approach for studying SOC dynamics.

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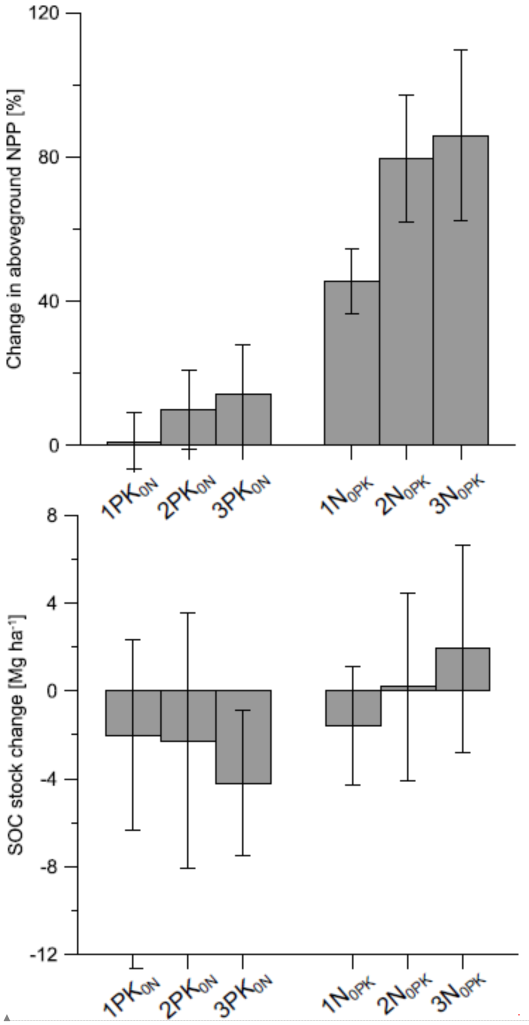
Table 1: The 10 experimental sites used in this study with coordinates [°N / °E], starting year (starting year in this study), year of last sampling, time span between first and last sampling [years] and length of the rotation [years].

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

Table 2: Basic soil parameters and climate data for the 10 study sites at the beginning of the experiments: clay and sand content [%], soil organic carbon content [%], soil pH, bulk density [g cm^{-3}], 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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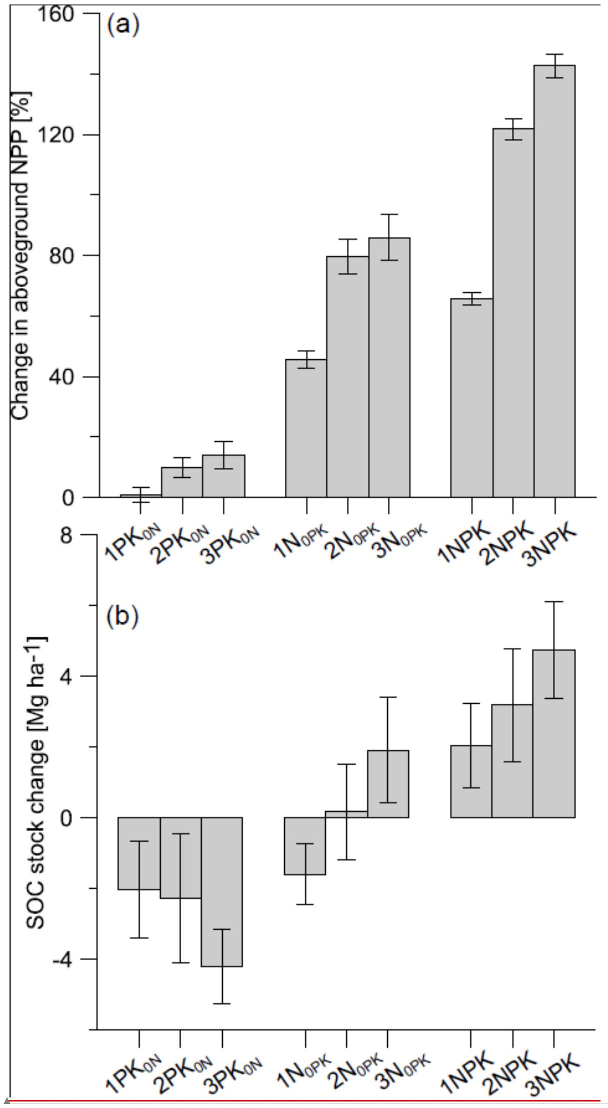

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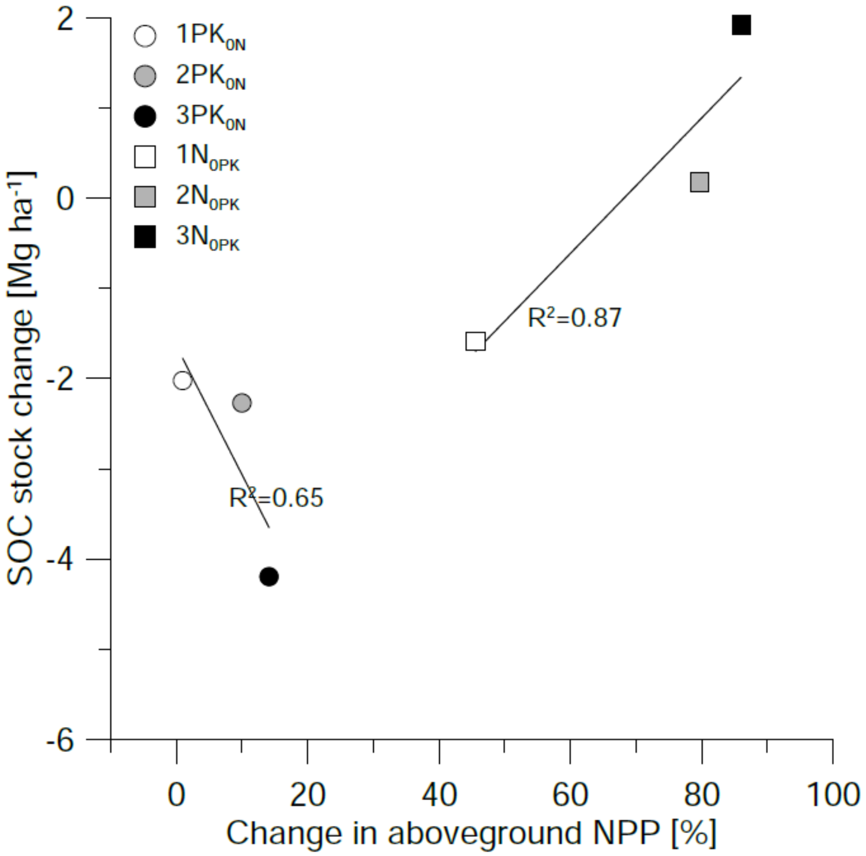
Figure 1: ~~A~~a) Average relative yield differences and ~~B~~b) Average soil organic carbon (SOC) stock differences between fertilised and unfertilised soils for all investigated levels of fertilisation (PK$_{0N}$, N$_{0PK}$, NPK) with standard ~~deviations~~errors.



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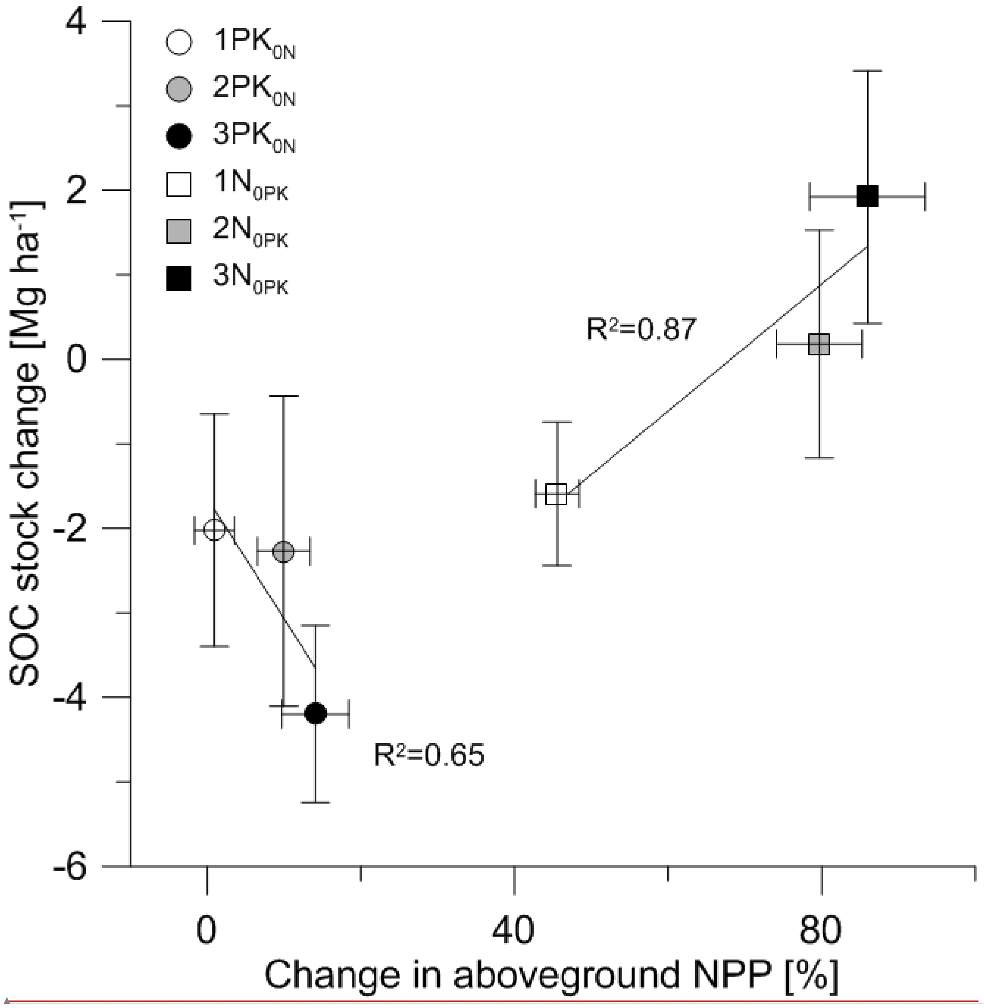

Figure 2: Correlation of average SOC stock differences between fertilised and unfertilised soils and average yield differences in fertilised and unfertilised soils with regression lines for the P and N fertilised regimes separately. Error bars indicate standard errors.

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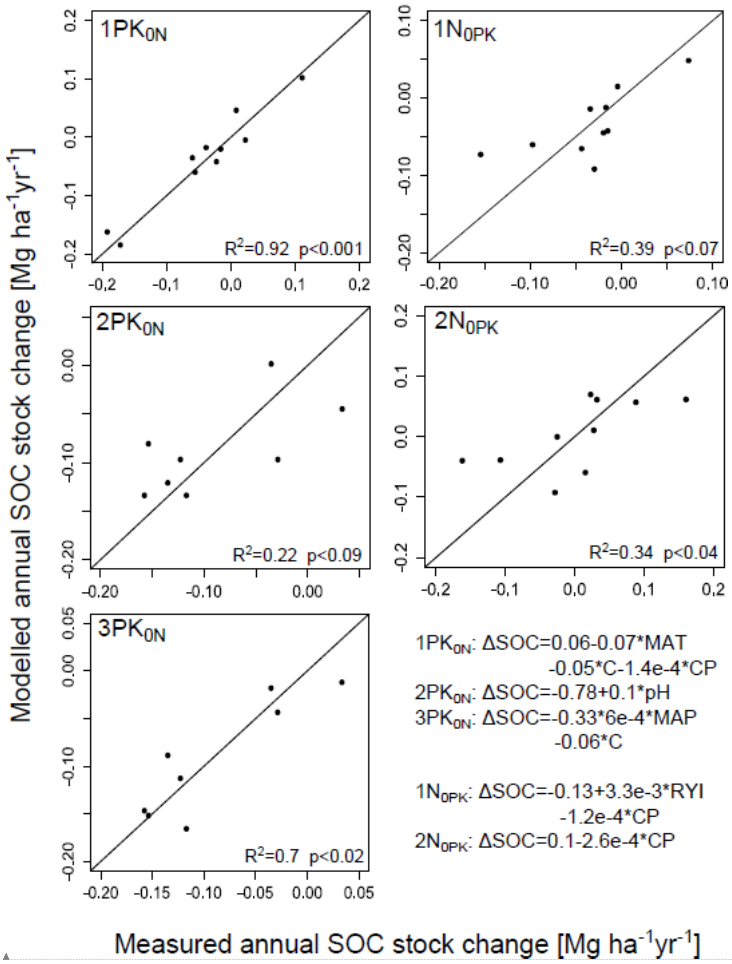

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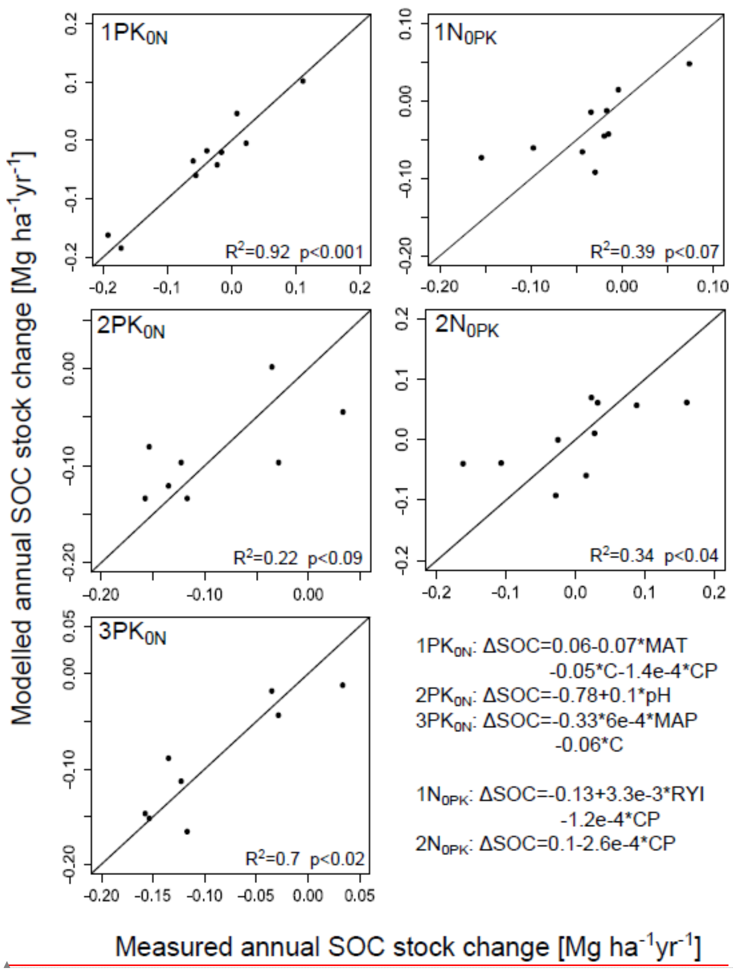

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Figure 3: Comparison of measured and modelled annual soil organic carbon (SOC) stock change [Mg ha^{-1}yr^{-1}] for all phosphorus-potassium (PK) levels and two N levels, with model equations in which MAT is mean annual temperature [°C], MAP mean annual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