

Extractability of Carbon, Nitrogen, and Phosphorus in United States Grasslands

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Abstract

Tracking how energy flows within and across ecosystems is imperative for understanding interactions among biogeochemical cycles. Aquatic ecosystem metabolism is inextricably linked to the terrestrial landscape, with many lakes getting over 50% of their carbon from terrestrial sources. Nonetheless, there are few large scale measurements of actual carbon export from terrestrial ecosystems. Instead, scientists have relied on a mass balance approach to estimate the quantity of carbon coming into aquatic ecosystems based on estimates of riverine carbon delivery to the ocean. This approach has left many unanswered questions related to the controls on terrestrial organic matter export, both in terms of quantity and quality. Here I used Water Extractable Organic Carbon (WEOC) to estimate potential terrestrial carbon export and to understand the mechanisms controlling these exports. Results from extractions performed at 19 grassland sites across the United States suggested that 1-5% of their total soil carbon was in the water extractable organic carbon pool. In addition, this work suggested that soils selectively retained nitrogen and phosphorus, with less organic nutrient export relative to organic carbon to aquatic ecosystems. These data demonstrated the usefulness of measuring water extractable organic matter (WEOM) on broad spatial scales to gain a better understanding of both the amounts and types of organic matter that are available for export from terrestrial ecosystems.

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Introduction

Carbon has been referred to as a “unifying currency” because it is so central to the activities of life and energy on our planet (Prairie and Cole 2009). Sunlight fuels the conversion of inorganic carbon into organic carbon, which provides energy and structure for organisms on Earth. Through this lens, we can view the transformations and flows of various carbon compounds as an overarching integrator of biological and ecosystem level processes. By better understanding how carbon moves from one place to another, and how it is transformed along the way, we may better understand the connections among ecosystems.

Until relatively recently, inland waters such as lakes and rivers have largely been ignored in the global carbon cycle (Cole et al. 2007; Tranvik et al. 2009). We have long known that rivers transfer large amounts of carbon from the landscape to the ocean (Schlesinger and Melack 1981; Meybeck 1982), but the simplifying assumption was that inland waters acted as a passive transport system. The basis for this assumption was the idea that organic matter transported from terrestrial ecosystems is aged and should be relatively resistant to microbial degradation. However this assumption has been challenged by findings of carbon dioxide supersaturation in lakes (Cole et al. 1994) and respiration in inland waters often exceeding primary production (del Giorgio and Peters 1993; del Giorgio et al. 1997). These findings piqued a new interest in understanding the importance of terrestrial subsidies to aquatic ecosystems and many studies have found significant terrestrial contributions to aquatic carbon budgets (Jansson et al. 2000; Biddanda and Cotner 2002; Pace et al. 2004; Lennon and Pfaff 2005; Cole et al. 2011;

Cole and Solomon 2012; Karlsson et al. 2012; Wilkinson et al. 2012; Wilkinson et al. 2013).

We now know that the “passive pipe” model for understanding aquatic ecosystems is inadequate and that in fact both lakes (Cole et al. 2007; Tranvik et al. 2009) and rivers (Raymond et al. 2013) transform significant amounts of carbon on the path from land to ocean. While the estimates of carbon delivered to the ocean have changed very little over the years (Aitkenhead and McDowell 2000), we have greatly improved our understanding of carbon transformations in inland waters before they reach the ocean (Schlesinger and Bernhardt 2013b). Tranvik and others (2009) suggested that lakes may emit as much as 0.54 Pg of carbon to the atmosphere annually and Raymond and others (2013) estimate the annual export from streams and rivers to be 1.8 Pg. These estimates would mean a total of nearly 2.4 Pg of C exported to the atmosphere from inland waters, or nearly three times more carbon than is exported from freshwaters to the oceans annually (Schlesinger and Bernhardt 2013a). This number is also of the same order of magnitude as human carbon dioxide emissions due to fossil fuel burning. When wetlands are included as part of inland waters, the total carbon dioxide outgassing from freshwaters could be as high as 3.28 Pg (Aufdenkampe et al. 2011). Interestingly, this analysis by Aufdenkampe and others (2011) has a substantially lower estimate for global emissions from streams and rivers than more recent analysis (0.56 Pg vs 1.8 Pg). If the stream and river estimate from Raymond and others (2013) is combined with the lake and wetland estimates by Aufdenkampe and others (2011), it suggests a global emission of ~4.5 Pg C from freshwaters. If outgassing of carbon dioxide in inland waters is truly this

high, it also means that current estimates for the annual export of carbon from terrestrial ecosystems are likely too low. Therefore, it is imperative that we continue to refine our understanding of aquatic carbon processing and terrestrial export to reconcile these differences.

Despite the abundance of research demonstrating degradation of terrestrial organic matter (TOM) in aquatic ecosystems, direct measurements of actual TOM export are sparse. In part this is due to practical difficulties, with most current estimates using streams and rivers as integrators of watershed export, but these estimates may underestimate the original terrestrial contribution because of processing that has already occurred in the streams before sampling. An alternative approach would be to utilize a network of seepage meters at the land-stream interface of 1st order streams in order to quantify the amount DOM loaded into aquatic ecosystems. This approach presents its own drawbacks though, as developing a network of meters that could lead to a meaningful data set on continental and global scales would be labor intensive and may underestimate terrestrial export during storm events when overland flow is an important pathway for organic matter transport (Brooks et al. 2012).

To address the lack of direct TOM export measurements, I have proposed an alternative method to the two previous described. By measuring water extractable organic matter (WEOM) at 19 grassland sites across the United States, my goal was to set an upper estimate for the TOM export from USA grasslands. While this measurement represents only the potential TOM export and is not a direct measure of what actually ends up in aquatic ecosystems (likely an overestimate of the actual export value), it

represents a novel estimate for terrestrial export and helps define a range of values to better understand the magnitude of TOM export on a large spatial scale.

Methods

Study Sites and Soil Collection

For this study, soil samples from 19 grasslands sites across the United States were used for WEOM extractions (Figure 1). Each site (defined as a unique geographic location) is part the Nutrient Network, a global network of grassland ecosystems (Stokstad 2011) consisting of over 70 experimental sites globally. Soils used in this study were collected the year that each site joined the nutrient network, prior to administering any of the nutrient network treatments. At the time of the extractions, soils were between 1-3 years post collection. Within each of the nutrient network sites, I extracted organic matter from soils in 10 plots (defined as an independent sub-unit within a site). The Nutrient Network sites provided a broad spatial scale with a consistent data collection protocol (Borer et al. 2014). As part of their experimental protocol, all nutrient network sites measure a host of soil chemistry and climatic variables (Borer et al. 2014), providing ancillary soil data to WEOM extraction values (Table 1). In addition, each site was assigned a soil texture classification based on the relative abundance of sand, silt, and clay particles using a soil texture triangle from the United States Department of Agriculture (USDA, Soil Texture Calculator).

Extracting Organic Matter

WEOM was extracted using cold water (45 ml of ultra-pure water from a NanoPure™ system; Thermo Scientific) and 1 g dry weight of air dried soil. The solution was shaken at 200 RPM for 48 hours at 4°C. After extraction, samples were filtered

through Whatman ® GF/F filters (nominal pore size ~0.7 µm), that had been pre-combusted at 500°C for 4 hours. Filtrate was collected in combusted (500°C for 5 hours) glass vials and stored at 4°C until analyses were performed.

Quantification of WEOM

To quantify WEOM, I measured dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP). For DOC analysis, filtrate from the WEOM extractions was acidified to pH 2 using concentrated hydrochloric acid. Each sample was analyzed using a Shimadzu TOC-V combustion auto-sampler to measure both DOC and TDN using potassium hydrogen phthalate and potassium nitrate as reference standards. TDP was quantified using the molybdenum blue spectrophotometric method (Murphy and Riley 1962).

Calculations and Statistical Analysis

Comparisons of nutrient extractions by soil texture (Figures 2, 3, 4, 5) were analyzed using a MANOVA approach, treating extractable nutrients as regression points with categorical predictors. In each instance, the null model was constructed to make mean values of extractable nutrients equal across soil textures, thereby assuming that soil texture had no effect on nutrient extractability. An alternative model allowed the nutrient extractability to vary at one or more soil textures. These model outputs were compared using an F-test to determine if soil texture was a significant predictor of nutrient extractability. In the case of significant relationships, subsequent univariate tests were performed to determine which variables were affected by soil texture. All other analyses used simple linear regression to test for significant slope parameters between numeric

variables. Analyses with outliers excluded are clearly identified in the figure legends. All statistical analysis and data visualization were done in JMP Pro10 (SAS Institute Inc., Cary, NC).

Results

Quantifying WEOM

Water extractable organic carbon (WEOC) varied from 0.80% of the soil carbon pool to 5.20% with a mean value of 2.27% across all 19 study sites. Water extractable nitrogen (WEN) showed a similar range with minimum, maximum, and mean values of 0.66%, 3.21% and 1.80% respectively. Of the 5 unique soil textures analyzed, sandy soil had the largest proportional potential export for both carbon and nitrogen with mean values of 4.58% and 1.82% respectively (Figure 2). Soil phosphorus was much less extractable, with extractability ranging between 0.029 and 0.595% (Figure 3). Mean phosphorus extractability was lowest in sandy soil (0.087%) and highest in silty loam soil (0.321%) but showed the largest range in extractability in sandy loam soil (Figure 3).

Nutrient ratios of WEOM

C:N and C:P ratios were calculated as total dissolved organic C (TOC) : total dissolved nitrogen/phosphorus (TDN/TDP). Mean C:N (mol: mol) of WEOM by soil texture ranged from 14.0 to 21.7, while the soil C:N varied from 13.2 to 17.6 (Figure 4). Across all soil textures, WEOM C:N ranged from 8.3 to 52.6 (Figure 4), with a median C:N of 15.9. Soil C:N showed less variability with a range of 9.1 to 23.4 (Figure 4) and a median value of 13.9. C:N in the extracted portion was significantly higher than the soil C:N in all soil textures except for silty loam (Figure 4). This suggests that potential export from grassland systems is nitrogen poor relative to the soil themselves. In contrast,

C:P in WEOM was lower than the C:P of the soil it was extracted from at all sites, indicating that extracted organic matter is phosphorus-rich relative to the parent soil. Mean C:P of WEOM by soil texture ranged from 116.5 to 508.4 and soil OM varied from 253.3 to 7183.1 (Figure 5).

Soil Characteristics Correlated with Nutrient Extractability

A recent study suggested that lake pH may be an important predictor of the contribution of terrestrially derived organic matter to aquatic carbon budgets (Wilkinson et al. 2013). In their study, Wilkinson and others found that the fraction of particulate organic matter (POM) derived from terrestrial sources significantly increased with more acid lake pH across a 39-lake survey. Therefore, we analyzed the changes in potential carbon export from grassland soil of varying pH to test if soil pH had any effect on the extractability of soil carbon. The 190 plots used in our analysis ranged in pH values from 4.8-8.4. Across this pH gradient, WEOC was negatively correlated with soil pH (Figure 6), suggesting that more acidic soil could potentially export more carbon than more neutral soils. Interestingly, the C:N ratio of extractable organic matter was not significantly correlated with pH (Figure 7), suggesting both carbon and nitrogen content varied in a similar way with respect to changing pH. However, the C:P of the extractable matter showed a negative trend with pH (Figure 7), indicating that more acidic soils could potentially export phosphorus-poor material. In addition I examined correlations between soil cation concentration and nutrient extractability to test for the potential importance of interactions with minerals in limiting nutrient export. Carbon and nitrogen

showed decreasing extractability as soil cation concentration increased whereas phosphorus extractability showed the opposite trend (Figure 8).

To understand soil characteristics that relate to dissolved phosphorus export, I looked at phosphorus extractability and stoichiometry across a productivity and soil C:N gradient. Along a productivity gradient, C:P of the extracted material was positively correlated with NPP ($p=0.0005$, Figure 9) suggesting that as productivity increased, exported material was increasingly phosphorus poor relative to carbon. In addition, carbon and nitrogen extractability decreased as soil C:N increased ($p < 0.5$, Figure 10) but phosphorus extractability increased marginally along the same soil C:N gradient ($p=0.0872$, Figure 10).

Discussion

The results of this study demonstrate two key points. First, soil pH and cation concentration are important for understanding how dissolved carbon, nitrogen, and phosphorus may be retained in soil. This study shows that carbon extractability was related both to soil pH ($p < 0.0001$, $R^2 = 0.18$; Figure 6) and base cation strength ($p < 0.0006$, $R^2=0.06$; Figure 8), suggesting adsorption to mineral particles is likely a contributing factor in retaining dissolved carbon in topsoil, but these mineral processes have a less varied effect on the retention of dissolved phosphorus. Instead, the positive relationship between extracted C:P and productivity ($p = 0.0005$, $R^2=0.11$; Figure 9) suggests that biological processes such as primary production had a larger effect than did mineral sorption on the overall phosphorus extractability. Second, these results indicate that WEOC can provide an upper estimate for the quantity of exportable carbon from terrestrial ecosystems, thereby establishing a potential export range for understanding the

magnitude of carbon flows from terrestrial to aquatic ecosystems. By establishing this potential export range, we can begin to examine the mechanisms of carbon transformation that would account for retention of carbon in, or export from, terrestrial ecosystems.

Carbon retention in soil

In general, physical processes are thought to be the major mechanisms for carbon retention in soil (Kalbitz et al. 2000). For many years, the paradigm has been that depletion of DOC concentrations by sorption to particles in mineral soil is the major mechanism for immobilizing dissolved carbon, with up to 90% of DOC adsorbed when passed through a mineral soil matrix (Guggenberger et al. 1998). However, there is now a growing body of evidence suggesting that WEOC may be readily available for microbial utilization and therefore biological mechanisms for carbon retention may also be important (Guggenberger and Kaiser 2003), particularly in the surface soils where sorption is less significant (Guggenberger et al. 1998).

Studies addressing the bioavailability of WEOC have found a wide range of bioavailable WEOC with values ranging from single percentages of the WEOC stock (Kalbitz et al. 2003) to upwards of seventy percent (Gregorich et al. 2003). While an understanding of the controls of bioavailability that may result in such a wide range of values are still uncertain (Marschner and Kalbitz 2003), it is generally accepted that a typical WEOC sample will contain somewhere between 20-40% bioavailable material (Gregorich et al. 2003; Boyer and Groffman 1996; Embacher et al. 2007; Kalbitz et al. 2000; Kalbitz et al. 2003). Therefore, WEOC may be readily degraded by microbial

processing, even during high flow events (such as spring snow melt in the northern temperate zone) when water is being transported laterally through the topsoil. This would lead to the removal of carbon from the dissolved pool even when sorption processes may be limited. Furthermore, grasslands and farmland typically have much slower water infiltration rates compared to forested systems and therefore flow through topsoil is a more important hydrologic pathway for grasslands than for forests (Brooks et al. 2012). Furthermore, DOC during snow melt periods in the arctic, when overland flow is the primary flow path, have been shown to be more bioavailable than base flow DOC in these areas when the soil water percolates deeper into the soil (Michaelson et al. 1998). In this manner, biotic carbon retention may be a particularly important mechanism for capturing carbon prior to export in grassland systems during periods of overland flow.

While this study did not explicitly address the potential for biotic mechanisms of carbon removal in soil, it does provide insight on the role of mineral retention in topsoil. In contrast to previous assumptions, data from the present study suggested that soil mineral content is important for carbon retention, even in topsoil where previous work has demonstrated that mineral sorption was low. Carbon extractability was negatively correlated with cation strength ($p < 0.0005$, $R^2 = 0.06$; Figure 8), indicating that even in topsoil, mineral interactions likely have important effects on carbon retention. For instance, during periods of lateral water flow, the previous assumption may have been that sorption was not an important mechanism in removing carbon because of the low mineral influence in topsoil. However, this study demonstrates that if the flow pathway was from a soil with low cation strength (such as soil with granite parent material)

towards soil with higher cation strength (such as soil with limestone parent material), sorption may still be an important mechanism of carbon retention.

Sources of Dissolved Nitrogen and Phosphorus in Soil

In a broad sense, phosphorus and nitrogen come from very different sources. The nitrogen supply in the soil is ultimately largely derived from the atmosphere, with the biological process of nitrogen fixation transforming di-nitrogen gas into biologically available forms. In contrast, the mediation of phosphorus mobilization is geochemically rather than biologically mediated, coming from the weathering of phosphorus containing rocks. Furthermore, the relative solubility of nitrogen and phosphorus in soils are markedly different, with nitrate being readily soluble in water and phosphate tending to adsorb to soil particles, particularly charged clays. These fundamental differences in the sources and characteristics of nitrogen and phosphorus lead to different expectations for the mechanism structuring the water extractable fractions of these elements.

If we separate both nutrients into two major pools in the soil (a microbial biomass fraction and a soil organic fraction) we can then look at expectations for extractability from those two pools to infer what we might expect the water extractable fraction to look like. In both cases, we would expect the microbial fraction to be relatively insoluble, because nutrients stored in actively growing biomass should not be readily available for water extraction. Instead, the only extraction coming from this microbial biomass pool would be derived from cells leaking N or P and because both of these nutrients are often scarce, the expectation should be that cells would hold onto these nutrients tightly.

In the case of nitrogen, the expectation would be that the soil N pool would provide some amount of N to the water extractable portion (Curtin et al. 2006), consistent with our understanding of nitrate solubility. Therefore, the water extractable nitrogen (WEN) should be stoichiometrically similar to soil N pool if microbial activity is low and should be nitrogen depleted with respect to carbon if microbial activity is high because the WEN pool is reflective of the “leftover soil N”. In contrast, the expectation for phosphorus would be that the water extractable phosphorus (WEP) pool should reflect the microbial biomass pool of phosphorus. This is because phosphate has such a tendency to bind to particles (Brady and Weil 2002) that any phosphates left in the soil will likely be bound to soil particles. Therefore, the WEP pool should be very small and stoichiometrically similar to microbial biomass phosphorus pool because the major source of phosphorus to WEP would come from cell leakage. The data from this study, discussed further below, are consistent with this conceptual model of N and P dynamics and support the hypothesis that water extractable N and P likely differ in their source.

Nitrogen retention in soil

Relative to measurements of C:N export from global catchments, the WEOM C:N values measured in this study were slightly lower than what has been measured in the past (Alvarez-Cobelas et al. 2012) suggesting either that grasslands export nitrogen-rich material compared to other ecosystems or that selective retention of N occurs between the dissolution of soil organic matter and the eventual export of it into aquatic ecosystems. Globally, DOM exported in rivers has a C:N of greater than or equal to 20 (Alvarez-Cobelas et al. 2012; Meybeck 1982). This is much higher than both the global average for

soil of ~ 14 (Cleveland and Liptzin 2007; Meybeck 1982) and the average of the 19 study sites examined here (17.9, Figure 4), supporting a mechanism for removing nitrogen along the flow path to, or within, aquatic systems. The C:N of WEOM measured in this study tended to fall below 20 as well (Figure 4) suggesting that our estimate of potential organic matter export may be nitrogen-rich compared to the current estimates of what is actually exported in rivers. This, too, is consistent with the idea of nitrogen removal from the WEOM pool prior to delivery to the ocean.

To further explore this idea, I attempted to parse out an estimated C:N of grassland export. However, despite having a data set of over 500 measures of TOC export and over 900 measures of nitrogen export, Alvarez and others (2012) were unable to analyze the C:N of export specifically from grasslands because they lacked concurrent carbon and nitrogen measurements in this biome. Therefore, I examined C:N exported from temperate regions and found a mean C:N of DOC exported in the temperate regions ranged from 36-65 (Alvarez-Cobelas et al. 2012, supplemental figures), on the order of double the WEOM C:N found in this study. This difference suggests that WEOM undergoes significant nitrogen loss prior to export, which could occur either in the soils themselves or early on in the rivers.

At the global scale, soil C:N is typically much higher than the microbial biomass C:N, with values of around 14:1 and 8.5:1 respectively (Cleveland and Liptzin 2007). This fundamental difference in stoichiometry is consistent with the supposition that microbial growth would result in a drawdown of soil nitrogen relative to carbon. Our data

clearly support this mechanism, as the C:N of the extractable portion (presumably what is left over after microbial processing) tended to be nitrogen poor relative to the soil matrix.

On the other hand, preferential nitrogen removal is not always indicative of microbial processing. Nitrogen could be preferentially retained relative to carbon during sorption to mineral particles. If this mechanism is important, soil with higher mineral content should have less extractable nitrogen. For the 19 grassland sites examined here, nitrogen extractability decreased with increasing concentration of soil cations, lending support for mineral removal of nitrogen. Therefore, it is unclear whether mineral sorption or biological uptake was more important for nitrogen retention. What the data clearly demonstrate, however, is that nitrogen was selectively retained in the soil prior to export, by sorption and/or biological uptake.

Phosphorus retention in soil

Extractable phosphorus across all sites in this study was low, never exceeding one percent of the soil phosphorus pool. This is strong evidence that phosphorus is highly retained in soil. As with carbon and nitrogen, this retention could be a function of physical and/or biological processes. Indeed we know that interactions with clay particles and mineral components are both important for retaining phosphorus in soil and in lake sediments (Jacobson et al. 2000; Singer and Munns 1991). Interestingly, this study provided no evidence that soil mineral content impacted the extractability of phosphorus (Figure 8). This lack of relationship could be due to the fact that soil minerals are so effective at binding phosphorus that even at the lower end of soil mineral content examined here there was enough mineral strength to maximally bind phosphorus. In that

case, increasing the soil mineral content would have no effect on phosphorus retention and the low overall extractability of P supports this hypothesis. However, this study also provides other data indicating that microbial removal of phosphorus may be an important factor when considering the retention of phosphorus in grassland topsoil.

To explore the role of microbial processes, I examined phosphorus extractability across a number of soil gradients: pH, soil cation concentration, soil C:N, and productivity. Soil pH was chosen because it is a commonly measured soil parameter. Also, I examined the soil cation concentration which has been previously shown to be important for organic C retention in soil profiles (Baldock and Skjemstad, 2000). The concentration of multiple base cations was positively correlated with soil pH (Figure 11). Across this pH gradient, soil carbon was negatively correlated with pH ($p < 0.0001$, $R^2 = 0.18$; Figure 6) whereas phosphorus was not correlated with pH. There was a negative correlation between WEOM C:P and soil pH ($p < 0.0001$, $R^2 = 0.18$; Figure 7), demonstrating that the potential export pool had the lowest relative phosphorus content at the lowest pH. Furthermore, phosphorus extractability actually increased with increasing soil cation concentration (Figure 8) indicating more potential phosphorus release in soils with higher mineral content.

Another way to explore the relationship between phosphorus retention and microbial activity is by comparing across soil C:N. Using C:N as a signature of the microbial contribution to the organic matter pool (with low C:N, on the order of ~8-12 being more characteristic of microbial derived organic matter), I observed that phosphorus extractability increased slightly with C:N but this increase was not

statistically significant ($p=0.0872$, $R^2=0.02$; Figure 10). This was in contrast to carbon and nitrogen extractability, which both decreased with increasing C:N ratios (Figure 10). This suggests that high microbial activity is leading to more extractable carbon and nitrogen. The relationship of soil C:N and phosphorus is less clear, but certainly demonstrates that phosphorus is behaving differently than carbon and nitrogen. Given that the observed positive relationship between phosphorus extractability and soil C:N was only marginally insignificant ($p=0.0872$, $R^2=0.02$; Figure 10), other indicators of microbial activity are worth exploring.

Finally, WEOM C:P was positively correlated with site level NPP ($p=0.0005$, $R^2=0.11$; Figure 9) indicating that potential export from the most productive sites was increasingly phosphorus poor. These systems with high productivity may have a higher demand on phosphorus from actively growing plants, but also in systems with high plant productivity, microbial productivity is also likely higher due to similar controls on the two processes such as nutrients, light, and water availability. Therefore, the positive relationship between WEOM C:P and NPP also suggests a strong potential for a biotic mechanism for soil phosphorus retention.

The idea of preferential phosphorus utilization by actively growing microbial communities is not a new one. Retention of DOP in the upper layers of the ocean has been explained by the rapid and preferential incorporation of phosphorus from DOP into microbial biomass compared to that of DOC and DON (Clark et al. 1998; Paytan and McLaughlin 2007). This retention has the effect of limiting organic phosphorus export to the deep ocean and results in a distinct vertical profile of decreasing organic phosphorus

concentrations and increasing DOC/DOP at depth. While this mechanism differentially distributes phosphorus in the ocean, our data suggest a similar mechanism could control the horizontal transport of dissolved phosphorus in soils.

Using WEOC as an estimate of potential carbon export

This study suggests that grasslands across North America contain a significant fraction of WEOC, with 2%-5% of the total carbon pool being water extractable on a continental scale, in the same range as previous studies (Chantigny et al. 2010; Curtin et al. 2006; Gregorich et al. 2003). I evaluated the ability for WEOC to serve as an estimate for potential export by comparing calculated WEOC values for the Missouri River watershed to estimates of DOC export for the same area. The Missouri River watershed was used because it is a large watershed with a large portion of the watershed made up of grasslands. Published estimates range from 1.58 kg C ha⁻¹ yr⁻¹ to 60 kg C ha⁻¹ yr⁻¹ for DOC export from temperate grasslands, including some explicit estimates for DOC export from the Missouri River watershed (Table 2). Using an intermediate value of 10 kg C ha⁻¹ yr⁻¹, I estimated the annual export of DOC from the Missouri River watershed to be ca. 1.1 Tg C yr⁻¹ based on an area of 1.08 x 10⁸ ha (Malcolm and Durum 1976). Assuming an average soil carbon content of 146 MgC/ ha to a soil depth of 100 cm (West et al. 2013) with 1-2% WEOC (conservative estimate), I calculated a total of ~158-316 Tg of WEOC in the top meter of soil in the Missouri River watershed. Therefore, my estimated total DOC export from the Missouri River was on the order of 0.34-0.7% of the WEOC pool of this watershed. Including the full range of estimates for DOC export (1.58 kg C ha⁻¹ yr⁻¹ to 60 kg C ha⁻¹ yr⁻¹), the estimated total export would be 0.17 – 6.48 Tg C

annually as DOC. This full range then translates to DOC export representing 0.05%–4.1% of the Missouri River watershed WEOC pool.

The global carbon stock in soils is estimated as 1500 Pg C (Schlesinger and Bernhardt 2013a), and assuming 1%-5% WEOC, the global WEOC pool should be on the order of 15-75 Pg C. Therefore current estimates of terrestrial export to inland waters (2.4 - 4.5 Pg C as discussed in the introduction) would represent 3.2%–30% of the global WEOC pool, showing a little overlap with the upper end estimate from the Missouri watershed. One caveat to this comparison is that it assumes that current estimates of terrestrial export are entirely in the form of DOC, neglecting dissolved inorganic (DIC) and particulate organic carbon (POC) coming from land and thereby overestimating the actual percentage of WEOC exported as DOC. Therefore, the true percentage of the WEOC pool exported on a global scale is likely at the low end of the 3.2%–30% estimated range. To make a best guess at estimating the total terrestrial DOC export, we can apply the overlap of the two WEOC export percentage estimates (3.2%-4.1%) to the estimated global WEOC pool of 15-75 Pg C. This results in an estimated global annual DOC export from terrestrial systems of 0.48-3.08 Pg C, consistent with our current understanding of the global C cycle.

Certainly using WEOC as a measure of available carbon export has some limitations. One significant limitation is that it fails to take into account the role of precipitation in extracting the WEOC. Export of WEOM could depend on a number of different factors, but a major one would be the availability and movement of water to actually transport this organic matter. Intuitively, the frequency and intensity of

precipitation received at a given site could play a major role in determining that actual magnitude of WEOM loss from that site. While it seems reasonable that WEOC loss should be affected by changes in precipitation, the relationship between WEOC and precipitation is not a straightforward one, as the WEOC values in this study showed no significant trend with any measure of site level precipitation (data not shown).

Furthermore, WEOC measurements do not take into account how quickly or slowly WEOM may be exported. In other words, the timescale at which a WEOC may be an accurate measure of potential export remains unclear. Nonetheless, WEOC can provide a starting point for thinking about the amount of organic matter available for export.

Given that WEOC is simply a measure of readily leachable carbon, it is clear that this number should be larger than the amount of dissolved carbon that is actually exported into aquatic ecosystems due to the potential for soil to retain and metabolize some of this carbon through both physical and biological processes as well as differences in precipitation. Taken together, our knowledge of soil adsorption capacity and the increased understanding of the relatively high bioavailability of WEOC suggest that only a small percentage of the WEOC pool should actually be exported to aquatic ecosystems. This line of thinking is also supported by work that showed that 95% of the DOC in forest throughfall was removed prior to leaching into a stream (Qualls and Haines 1992). As previously discussed, sorption can remove upwards of 90% of the carbon from the WEOC pool in highly mineral soil and current estimates suggest that around half of the WEOC pool is readily available for microbes. Furthermore, this study demonstrates that sorption processes may be important in retaining carbon even in topsoil, a mechanism

that was not previously considered important. Therefore, it would be reasonable to assume that the amount of WEOC actually exported should be on the order of 10% or less.

Concluding remarks and future directions

Understanding the mechanisms involved in controlling the export of organic matter from terrestrial ecosystems is imperative for determine how these exports may change in the future. Inland waters receive major inputs of carbon, nitrogen, and phosphorus from the land surrounding them and therefore our understanding of the role of inland waters in these biogeochemical cycles must include a stronger understanding of the terrestrial-aquatic link. This study demonstrated that biological retention might be an important mechanism for retaining and structuring dissolved phosphorus in the soils and thereby limiting its availability for export to aquatic ecosystems. Nonetheless, both physical and biological mechanisms are important for carbon and nitrogen retention in topsoil. Since aquatic ecosystems receive large inputs of terrestrial matter during periods of high hydrologic flow (when lateral movement of water trough topsoil is more prominent), understanding the mechanisms of topsoil nutrient retention will add to our understanding of terrestrial subsidies to aquatic ecosystems.

While using WEOC as a measure of potential carbon export certainly has its limitations, it represents a novel upper end estimate for better constraining our understanding of terrestrial carbon exports to aquatic ecosystems. It sets an upper bound to potential carbon export, which can be used to provide evidence for the magnitude of processing that is occurring in soils prior to delivery to aquatic ecosystems. Also, it is

important to use these more mechanistic measures with less reliance on a mass balance approach for estimating terrestrial export. From this work, it is clear that WEOC is only a first step in estimates of carbon export as it is likely that only a small fraction of the WEOC is actually exported.

Future work should continue to narrow the range of terrestrial carbon export estimates by examining microbial processing and hydrology as key mechanisms for modifying and retaining WEOC before it is exported to an aquatic ecosystem. By better understanding the mechanisms controlling carbon export from terrestrial ecosystems we can further understand the role that terrestrial carbon plays in aquatic ecosystems and continue to uncover the fundamental linkages between land and water.

Table 1. Sampling site characteristics.

Each cell contains with mean value of the parameter at the given site, with estimates of one standard error included in parentheses.

Site Name	Soil Carbon as % Dry Mass	Soil Nitrogen as % Dry Mass	Soil pH	Mean Annual Temperature (°C)	Net Primary Production (g/m ²)	Mean Annual Precipitation (mm)
Boulder	1.25 (±0.07)	0.10 (±0.005)	6.86 (±0.129)	9.7	182.57	425
CBGB	0.59 (±0.05)	0.06 (±0.004)	6.15 (±0.040)	9	398.23	855
Cedar Creek	0.57 (±0.08)	0.05 (±0.006)	5.57 (±0.076)	6.3	198.36	750
Cedar Point	1.97 (±0.40)	0.14 (±0.016)	6.86 (±0.207)	9.5	248.26	445
Elliot	1.85 (±0.13)	0.15 (±0.008)	5.67 (±0.072)	17.2	370.01	331
Finley	3.32 (±0.12)	0.22 (±0.005)	5.21 (±0.023)	11.3	257.51	1104
Hall's Prairie	1.53 (±0.05)	0.14 (±0.004)	5.11 (±0.038)	13.6	741.14	1282
Hanover	4.86 (±0.19)	0.42 (±0.018)	5.07 (±0.045)	6.4	395.60	272
Hart	1.17 (±0.06)	0.10 (±0.004)	7.19 (±0.041)	7.4	101.00	1033
Lookout	16.18 (±0.55)	1.19 (±0.055)	5.04 (±0.050)	4.8	209.49	1898
Sagehen	8.88 (±0.89)	0.70 (±0.065)	6.11 (±0.111)	5.7	174.17	882
Sedge	2.31 (±0.12)	0.19 (±0.010)	6.84 (±0.037)	14.9	322.01	521
Sevilleta	0.32 (±0.02)	0.03 (±0.001)	8.23 (±0.056)	12.6	70.89	252
Shpstn Adler	2.63 (±0.20)	0.23 (±0.011)	7.88 (±0.083)	5.5	157.21	262
Sierra Foothills	2.37 (±0.10)	0.20 (±0.007)	5.93 (±0.050)	15.6	286.31	935

Site Name	Soil Carbon as % Dry Mass	Soil Nitrogen as % Dry Mass	Soil pH	Mean Annual Temperature (°C)	Net Primary Production (g/m ²)	Mean Annual Precipitation (mm)
Smith	6.27 (±0.37)	0.48 (±0.027)	6.04 (±0.073)	9.8	554.67	597
Spindletop	2.68 (±0.05)	0.26 (±0.005)	6.38 (±0.039)	12.5	454.14	1140
Trelage	3.58 (±0.11)	0.28 (±0.006)	5.84 (±0.106)	11	1408.13	982
Tyson	2.55 (±0.23)	0.23 (±0.017)	5.67 (±0.076)	12.5	479.21	997

* All measurements were made by participants in the Nutrient Network. Data were used with permission from participants in the Nutrient Network and obtained from Dr. Eric Lind (elind@umn.edu).

Table 2. Estimates of DOC exported from the Missouri River watershed and cool/temperate grassland ecosystems.

Estimated DOC export (kg C ha ⁻¹ yr ⁻¹)	Geographic Scale	Source of Estimate
1.58	Missouri watershed	Malcolm and Durum 1976
0-10	Mississippi watershed	Stets and Striegl 2012
10-60	Missouri watershed	Raymond and Oh 2007
4.9	Temperate Grasslands	Aitkenhead and McDowell 2000
10	Temperate Grasslands	Schlesinger and Melack 1981
56.56	Temperate Shrub land	Alvarez-Cobelas et al. 2012 (supplemental material)
40.57	Cold Temperate	Alvarez-Cobelas et al. 2012 (supplemental material)

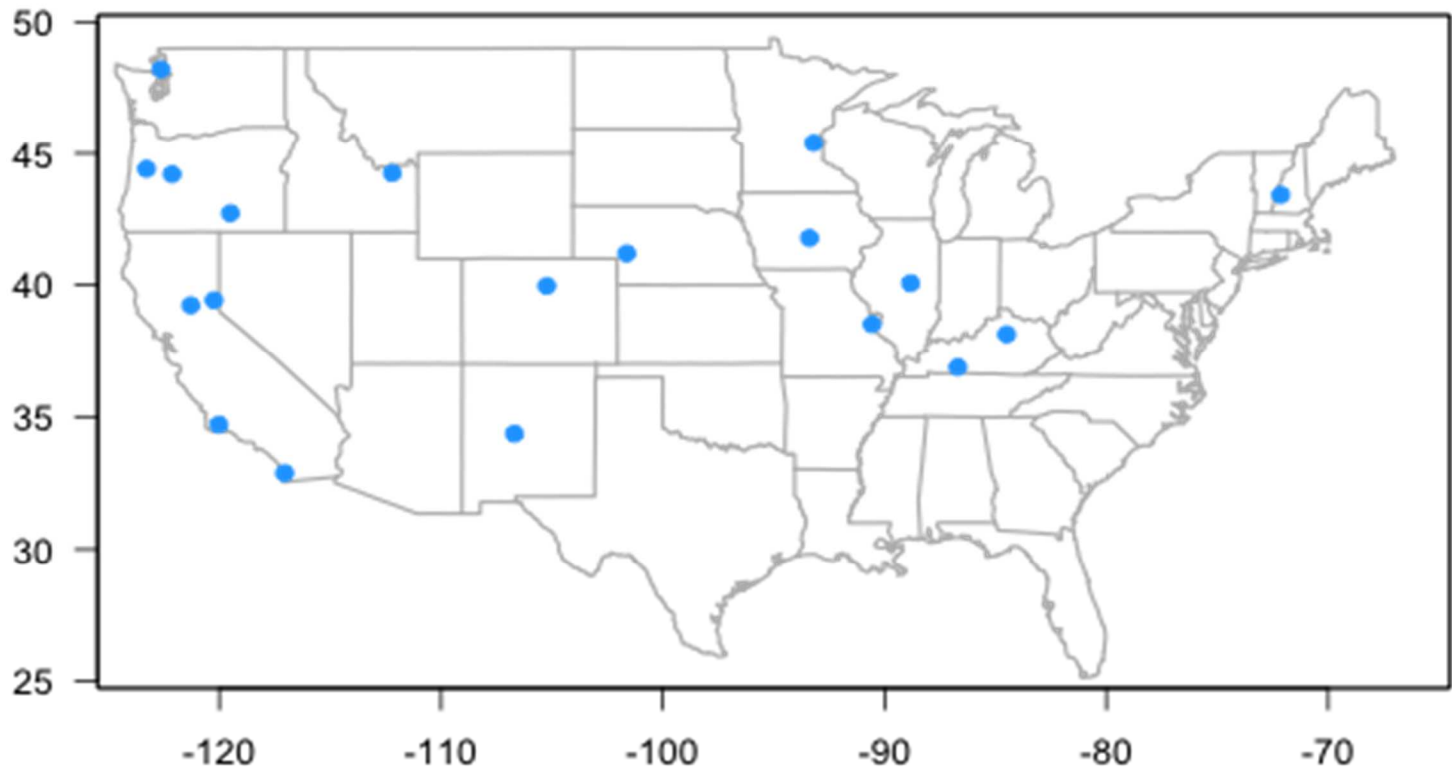


Figure 1. Map showing the location of each of the 19 sites used in this study.

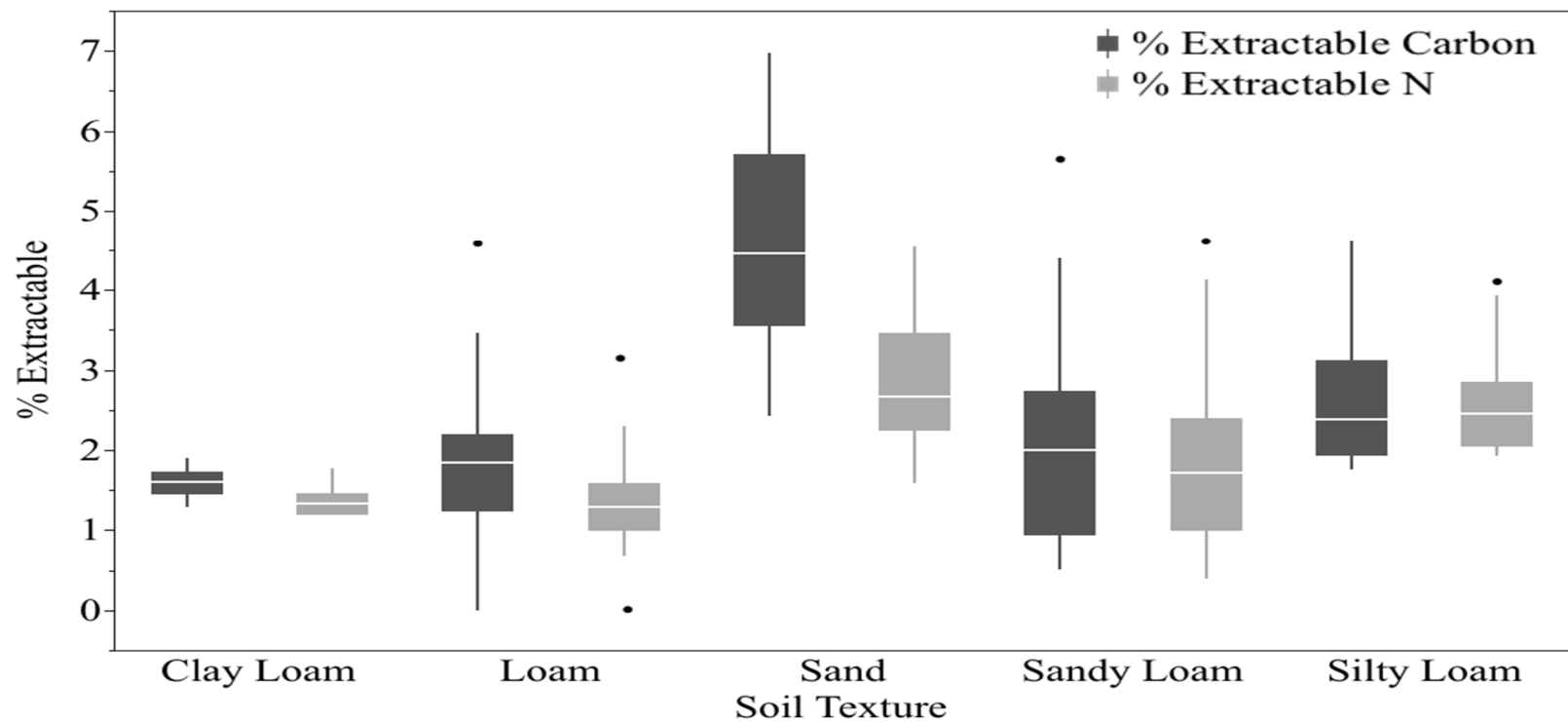


Figure 2. Extractable carbon and nitrogen (extracted/soil pool) by soil texture.

Both carbon and nitrogen extractability was significantly different across soils texture (manova using Wilks' Lambda, $p < 0.0001$ followed by univariate analysis). Significant differences between carbon and nitrogen extractability occur in loam and sand soils (paired t-test, $p < 0.0001$) and also in clay loam soils (paired t-test, $p = 0.0014$). These three soil textures showed a higher percentage of extractable carbon relative to nitrogen. Range for mean carbon extractability is 1.60% in the clay loam to 4.58% in the sand. For nitrogen, means range from 1.34% in Loam and 2.80% in sand.

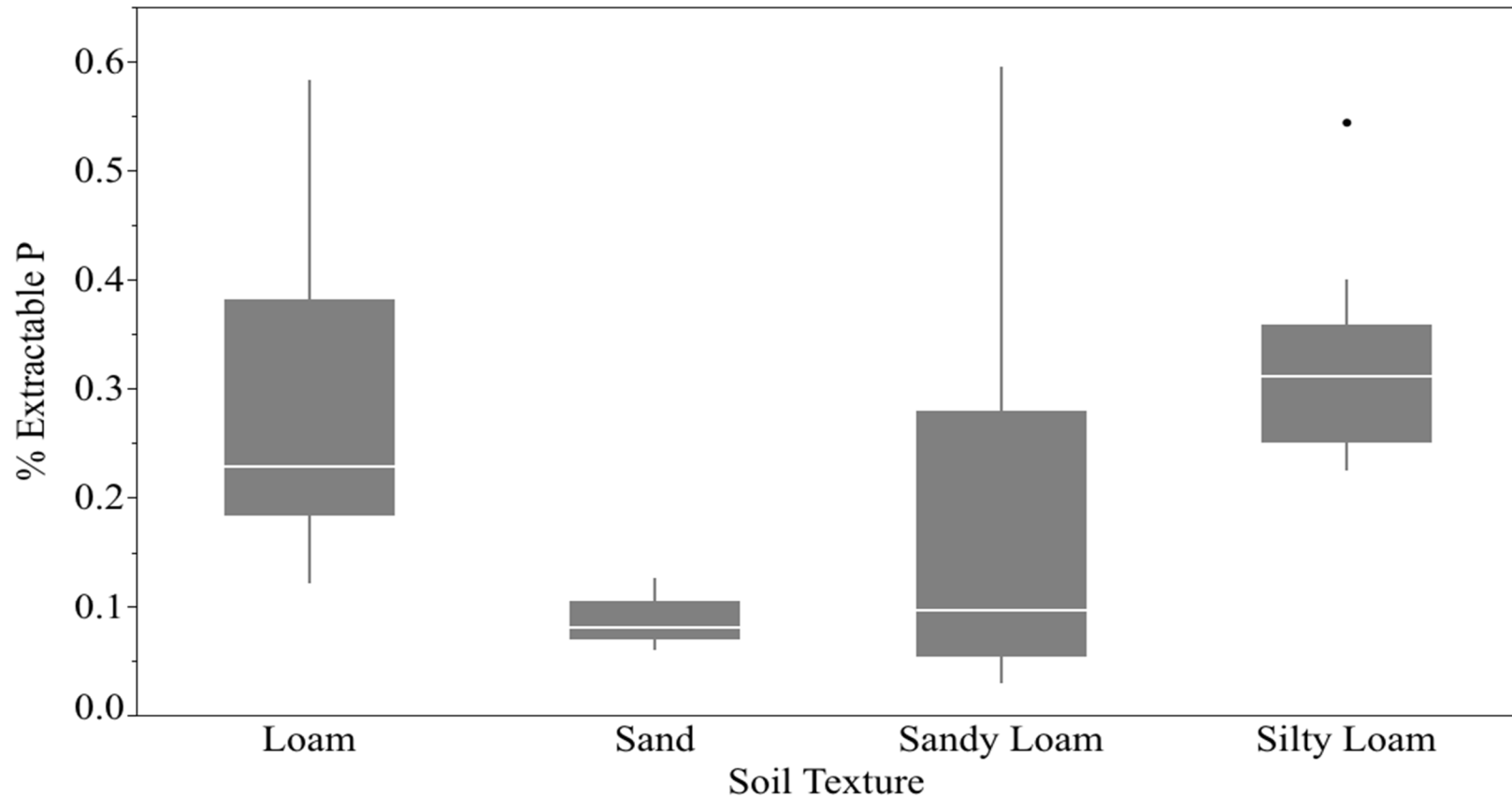


Figure 3: Extractable phosphorus (extracted/soil pool) by soil texture.

Phosphorus extractability was below 1% of the soil pool at all sites. Silty loam soil had the highest mean extractability at 0.32% and sand soil had the lowest at 0.09%.

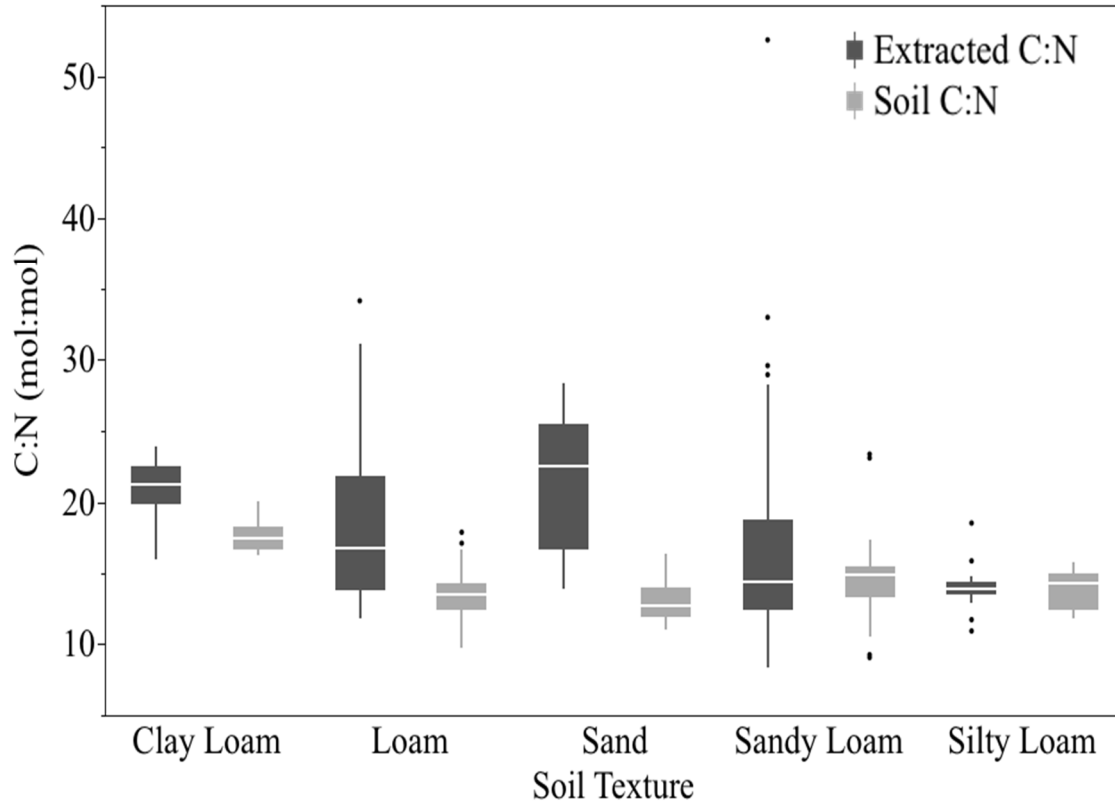


Figure 4. Comparison of C:N in the extractable fraction and parent soil material by soil texture.

Silty loam was the only soil texture where extracted C:N did not differ from soil C:N. In all other soil textures, the extracted C:N was significantly higher than the soil C:N (paired t-test, $p < 0.006$). Sandy soil showed the highest mean C:N ratio in the extractable fraction with a value of 21.7 but also had the lowest mean soil C:N ratio with a mean value of 13.1. Silty loam soil exhibited low mean C:N ratios in both the extracted fraction and the soil pool, 14.0 and 13.8 respectively. Clay loam soil showed the highest ambient soil mean C:N with a value of 17.6. For perspective, a global analysis done by Alvarez and others (Alvarez-Cobelas et al. 2012) found that the C:N of exported material was typically greater than 20. Thus our data show extracted values that would be on the lower end of what is expected of exported organic matter from world catchments.

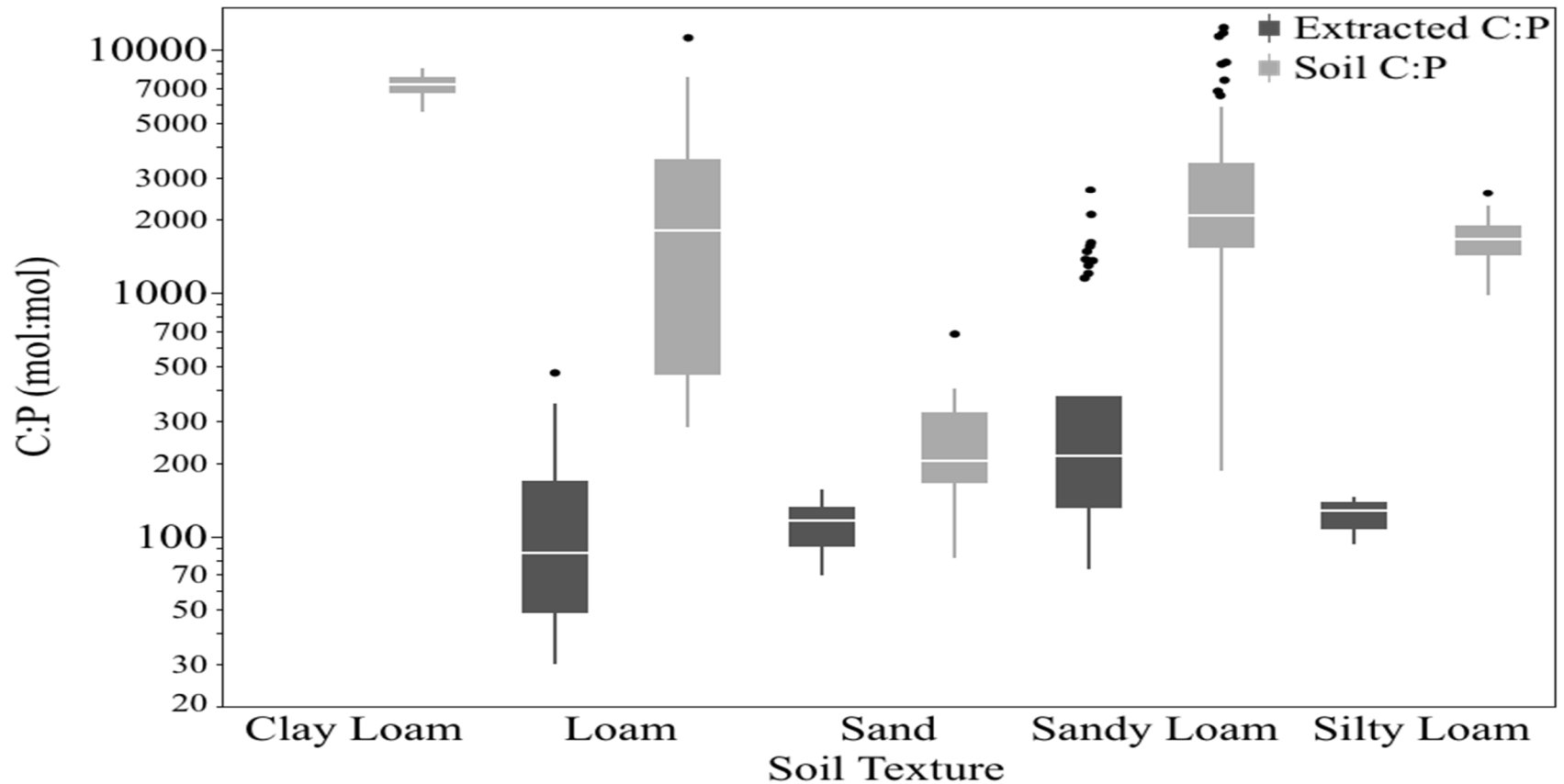


Figure 5: Box plots of C:P by soil texture.

Extracted phosphorus was not measured on any soil with clay loam texture so no extracted C:P could be calculated for this soil texture. For the other four soil textures, soil C:P was significantly higher than extracted C:P (paired t-test, $p < 0.0001$) and in some cases by more than an order of magnitude.

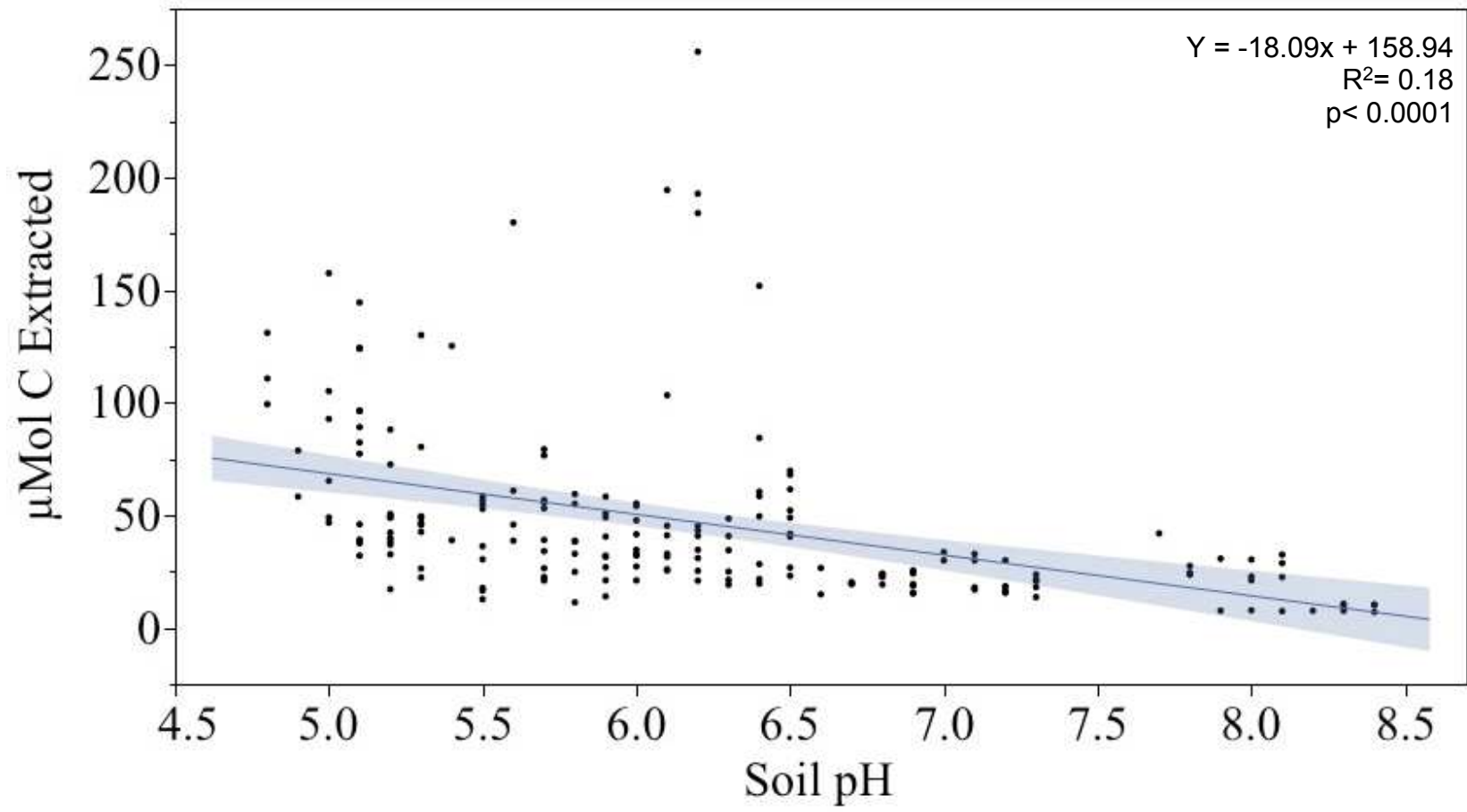


Figure 6: Carbon extractability across soil pH gradient.

Figure shows a significant decrease in the amount (μMol) of extractable carbon as soil pH increased. This supports the hypothesis that more acidic soil may export higher amounts of carbon to nearby aquatic ecosystems.

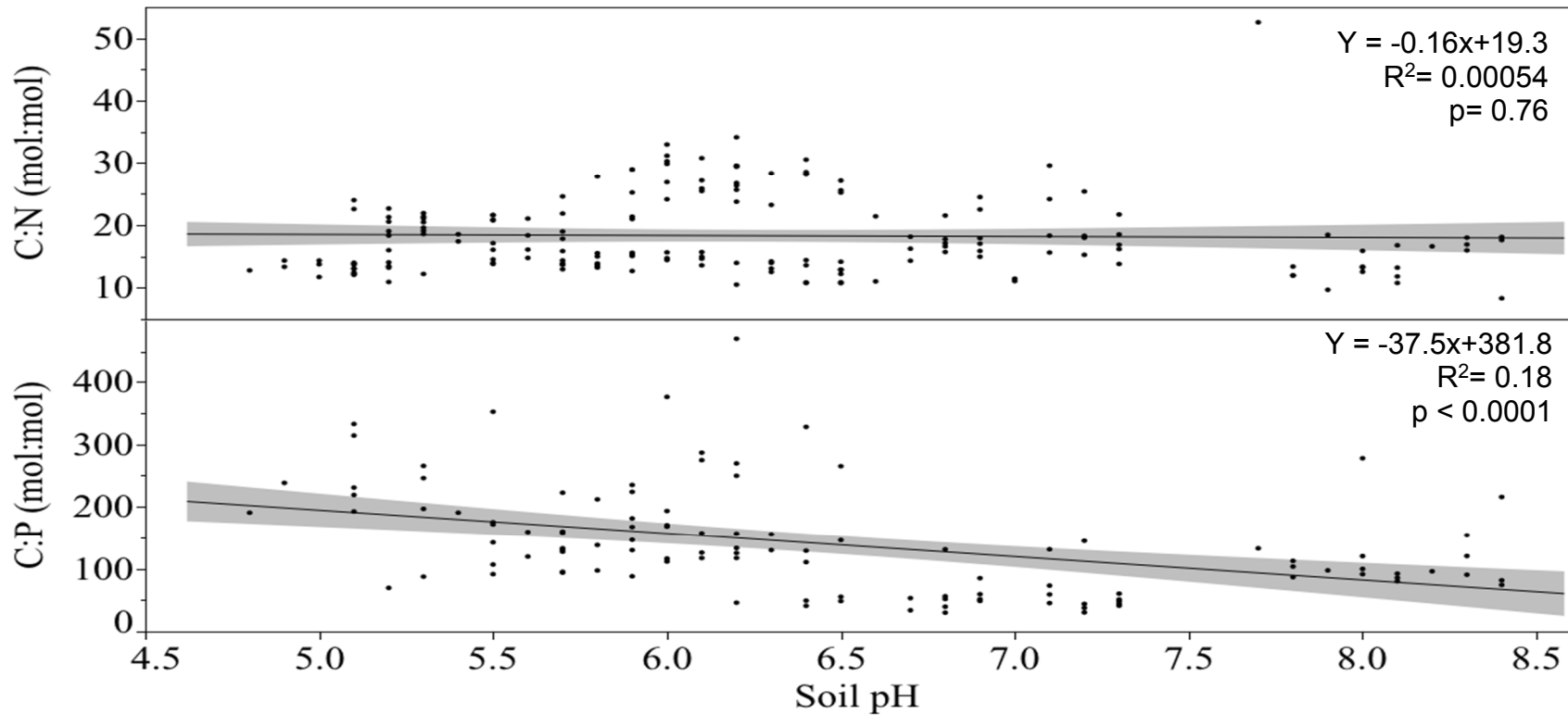


Figure 7: WEOM C:N and C:P across soil pH gradient.

WEOM C:N in the soil extractions shows no correlation to the soil pH, indicating the linear regression slope was not significantly different from zero. In contrast, WEOM C:P in the extracted fraction shows a significant decrease as soil pH increases. One site was excluded from C:P based on outlier status defined as the entire site (all ten plots) having values larger than the 3rd quartile plus 1.5 times the interquartile range. Exclusion of these outlier points doesn't change the basic relationship but significantly impacts slope leading to negative C:P at high pH, therefore they were omitted.

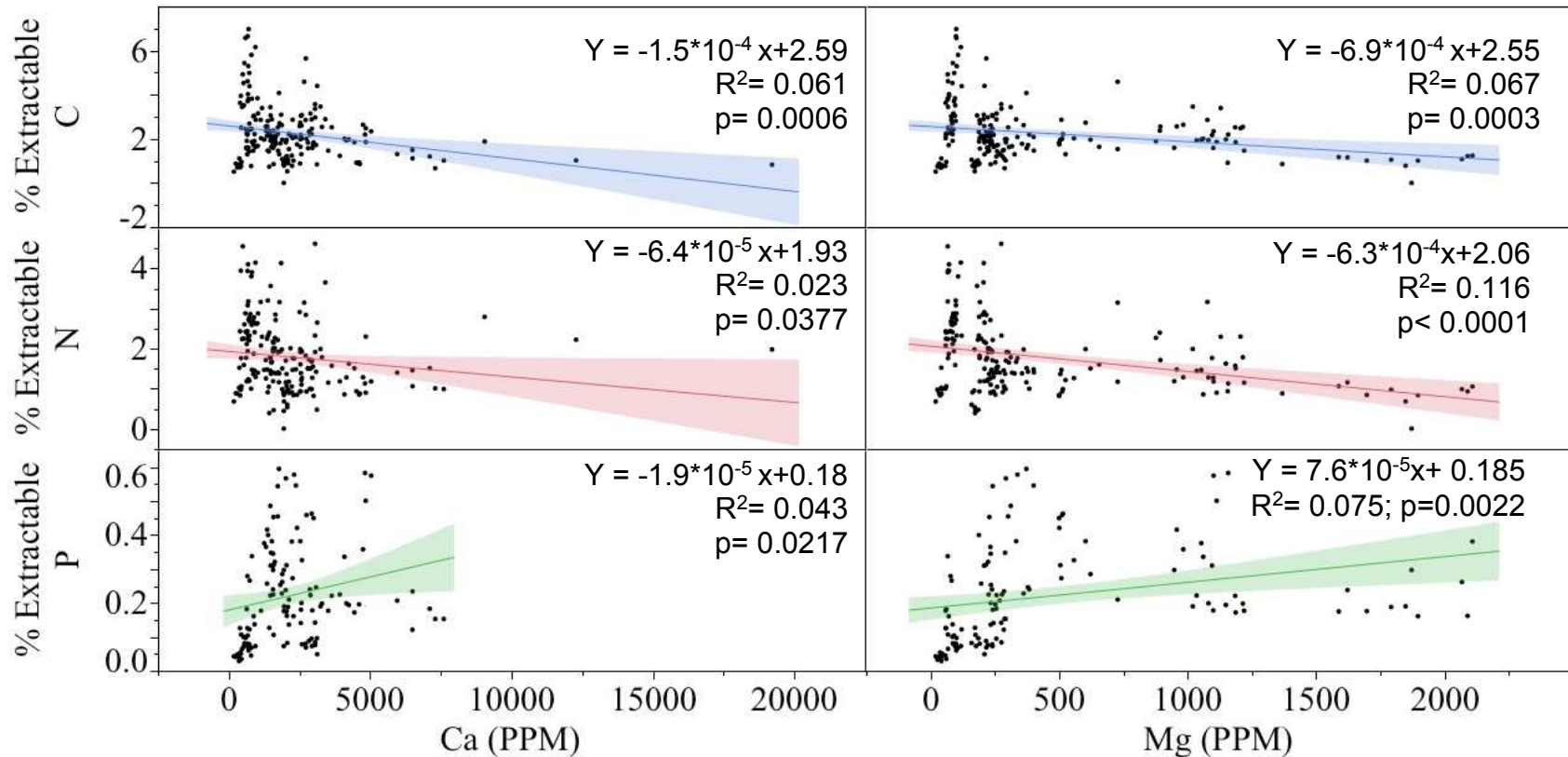


Figure 8: Extractable C,N,P and across soil cation strength gradient.

For both Ca and Mg, carbon and nitrogen show significant negative relationship whereas phosphorus shows a positive relationship. Base cation concentration was used as a measure of soil mineral content because it has been previously demonstrated to play an important role in carbon solubility and remineralization (Baldock and Skjemstad, 2000)

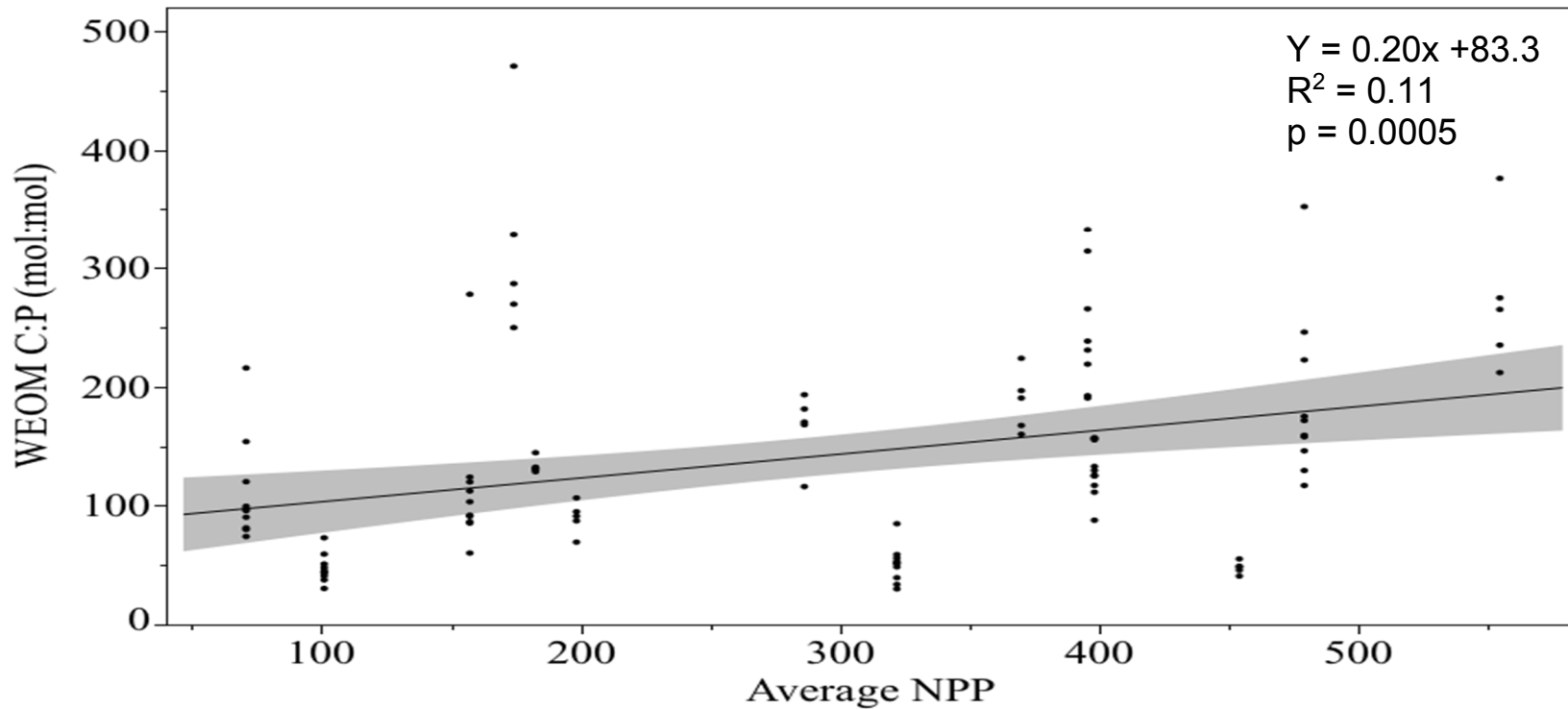


Figure 9: WEOM C:P by average site level NPP

WEOM C:P and average annual net primary production (NPP in g biomass/m²) were positively correlated at 14 of the sites ($p=0.0005$). One site was excluded from C:P based on outlier status defined as the entire site (all ten plots) having values larger than the 3rd quartile plus 1.5 times the interquartile range and an additional four sites lacked phosphorus data and could not be plotted. This suggests that the extracted material tended to be more depleted in phosphorus relative to carbon at high productivity sites. To calculate average NPP, annual NPP numbers were averaged at each site for all available years. Error bars are 1 standard error from mean.

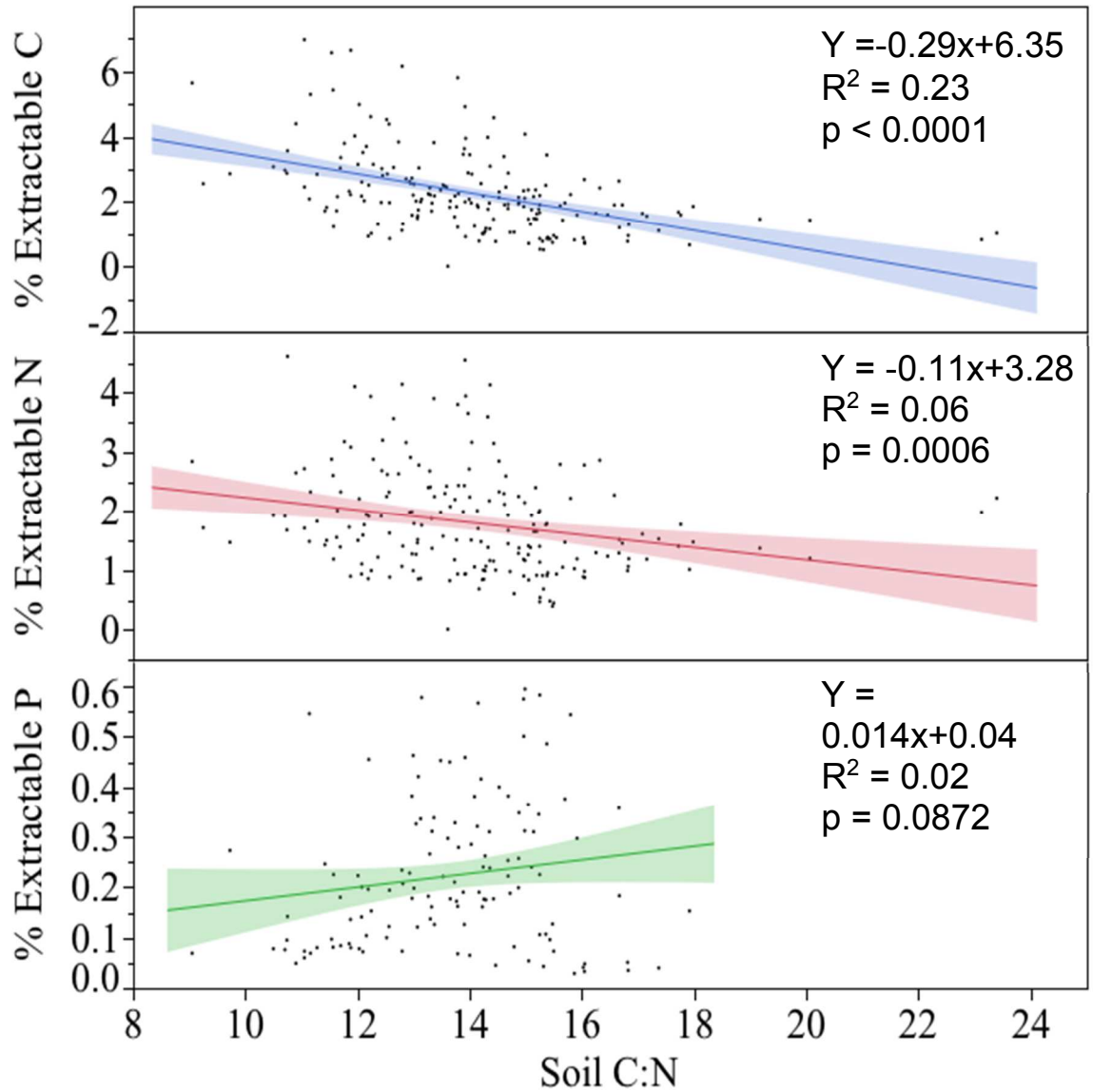


Figure 10: Relative extractability of carbon, nitrogen, and phosphorus across the soil C:N gradient.

Carbon and nitrogen were decreasingly extractable with increasing soil C:N ($p < 0.0001$, $p = 0.0006$ respectively). Conversely, extractable phosphorus was marginally insignificant but demonstrated an increasing relationship across soil C:N ($p = 0.0872$).

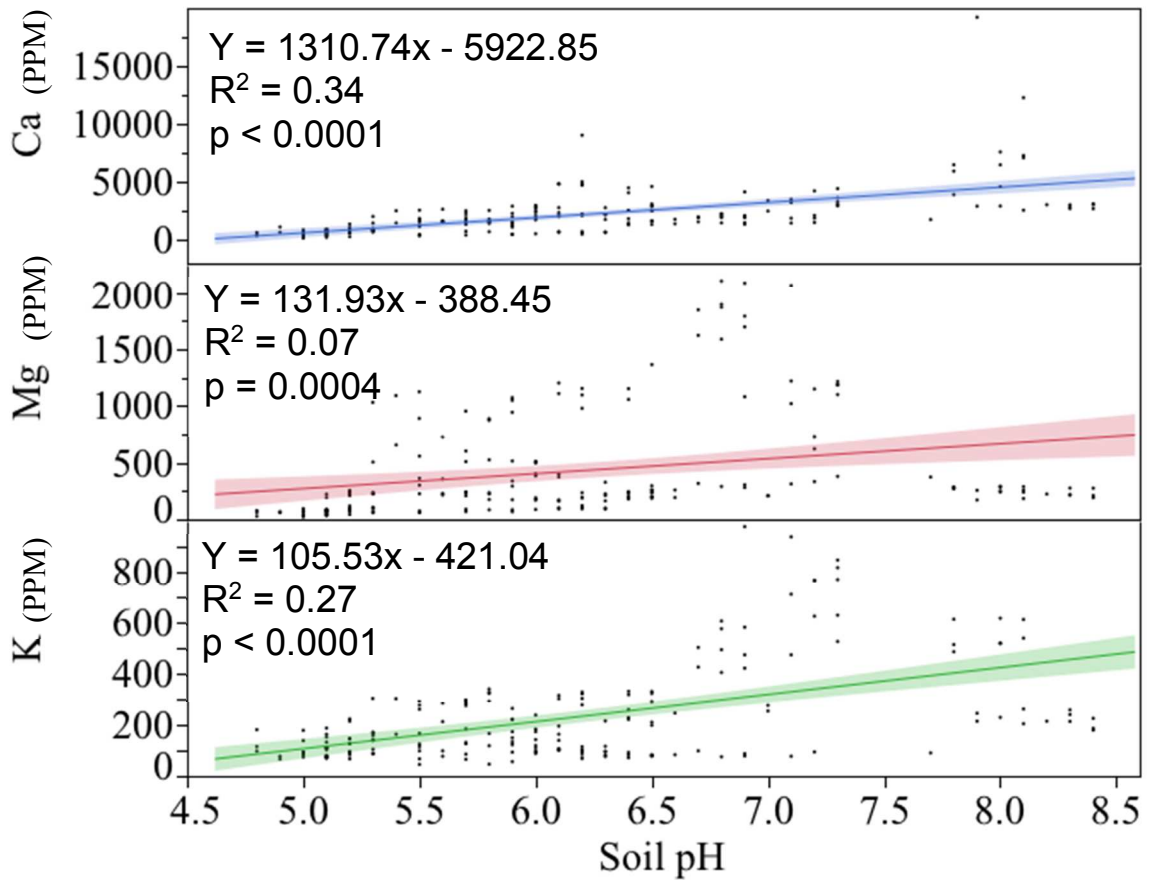


Figure 11: Soil cation strength across soil pH.

Figure shows that all cations showed a significant increase with increasing soil pH. This demonstrates that more acidic soils had lower base cation concentration than more basic soils, relating soil mineral surface to the commonly measured soil pH.

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