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Experimental Flooding Changes both Persistent and Bioavailable C, N and P in agricultural soil

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The Canadian Agri-Food Policy Institute
960 Carling Avenue, CEF Building 60
Ottawa, ON K1A 0C6
Telephone: 613-759-1027
www.capi-icpa.ca

Canada 



Abstract

Flood intensity and duration are expected to increase in Northeast Canada, increasing the amount of land, including farmland, that will experience flood events. Although much of the previous research focuses on the effects of flooding on the soil surface, flooding is also known to impact carbon (C), nitrogen (N) and phosphorus (P) dynamics in the rhizosphere, the region of soil influenced by root activity. Changes in C, N and P cycles can profoundly change ecosystem and agricultural productivity. Yet, it is unclear how C, N and P shift with flooding in agricultural land, given their complex interactions with soil minerals and the microbial community. This study examines two mechanistic controls on flooding -intensity and duration- through a controlled laboratory incubation experiment to better understand how different flood scenarios impact C, N and P cycles in agroecosystems. We found that both the stable, mineral associated, and the biologically available pools are susceptible to dramatic shifts with flooding. Flood duration had a greater impact on the stable, mineral bound pool, while flood intensity had more of an impact on the biologically available pool. However, C, N and P each responded differently to the various flood scenarios. We further found that the microbial community responds to increases in available substrates with greater levels of activity. These results show that increases in flooding can significantly change C, N and P dynamics in Canadian agricultural soil. Increasing perennial root biomass may be a simple way to lessen the effects of flooding. Roots increase microbial activity, allowing microbes to assimilate C, N and P into their biomass retaining them within the soil system. While it is impossible to prevent increases in flood duration and intensity, through understanding how flooding impacts soil C, N and P, farmers can build resiliency into their land before flooding changes the soil.

Introduction

With present and future climate change, much of the world will experience devastating shifts in weather patterns like increased flooding. Northeast Canada is predicted to experience warmer springs as well as greater winter and spring precipitation. These climatic changes are expected to induce greater spring snow melt in turn increasing peak flood intensity and duration (Jeong et al. 2014, Clavet-Gaumont et al. 2012). This change in flood pattern means that more land will be flooded annually, and that land will remain flooded for a longer period of time at a higher water saturation level.

Floods transform the soil environment, especially in the rhizosphere- the region of soil influenced by root activity. Much attention in understanding agricultural land's response to flood focuses on horizontal movement of surface soil. While less research focuses on how flooding impacts soil below the surface and through the soil profile. Water logging, caused by flooding, can drastically change the soil environment in the rhizosphere. Changes in soil flood dynamics are known to impact carbon (C), nitrogen (N) and phosphorus (P) cycles which are key elements for soil health and ecological functioning contributing to soil fertility (Gross et al. 2020, Luo et al. 2019, Miller et al. 2001). Yet, it is unclear how soil C, N and P respond to flooding, in terms of transformations or losses, due to their complex interactions with soil minerals, the soil microbial community and plant roots.

C, N and P are essential for soil health, strongly contributing to soil fertility and ecosystem productivity. Plants are reliant on soil N and P for growth and microbes are dependent on C, N and P for their growth and activity (Tian et al. 2017, Mori et al. 2017, Mooshammer et al. 2014). Thus, losses in soil C, N and P can decrease ecosystem productivity. For agroecosystems this may mean lower yields and a larger reliance on N and P fertilizer. Further, soil C is an essential global C sink, containing approximately 2-3 times the amount of C than is currently in the atmosphere (Paustian et al. 2016). Small changes in the soil C pools can cause dramatic increases in atmospheric carbon dioxide (CO₂) accelerating

climate change. Additionally, high losses of soil N and P into nearby water bodies can act as a pollutant, contaminating drinking water and increasing eutrophication (Zhang et al. 2018, Jalili 2009).

In Quebec, farmland is vulnerable to climate induced changes in flood dynamics. In 2016, there were 8.1 million acres of farmland in Quebec, of which 4.6 million was cropland (Statistics Canada, 2017). Given the large area of farmland in the region it is essential to determine how flooding specifically affects agroecosystems. Changes in flooding is especially relevant for agricultural land along the St. Lawrence River, which already is experiencing an increase in annual flooding (Larue 2019). This change in flood dynamics can not only degrade soil quality but also water quality in the St. Lawrence River and the flora and fauna reliant on it.

Agroecosystems may be particularly vulnerable to flood induced shifts in C, N and P as they already experience significant disturbance and may be less resilient as a result. Fortunately, unlike natural systems, agroecosystems can be directly managed to target greater resiliency during floods to help increase C, N and P retention. Essential for this retention is determining how different flood scenarios affect soil C, N and P dynamics on a mechanistic level.

Length and intensity of flooding may impact how C, N and P are potentially retained in or lost to the soil system. Increases in flood duration can cause greater water infiltration and logging as there is more time for the water to leach throughout the soil profile compared to shorter flood periods. Under low intensity flooding, less of the soil may become saturated compared to a high intensity flooding even during long flood duration periods. Increases in infiltration and the resulting saturation may lead to more of the rhizosphere to experience transformation of C, N and P. Thus, given how differing flood scenarios may impact the movement and transformation of C, N, and P it is necessary to examine the effects of flood duration and intensity independently and interactively. Currently, it remains

unclear how differences in these flood characteristics impact C, N and P dynamics on a mechanistic level in agricultural systems.

While previous studies show that the fate of C, N and P are linked during flood events (Gross et al. 2020), little is known about how flooding impacts C, N and

P dynamics in agroecosystems. This study provides novel insight on soil C, N and P shifts in agricultural soil during and following flood events by examining two potential mechanistic controls: flood intensity and duration.

Relevant Background Information

Most soil C, N and P is bound to soil mineral surfaces and thus largely biologically unavailable for plants and microbes (Gross et al. 2020, Gross et al. 2018, Keiluweit et al. 2015, Kaiser and Kalbitz 2012, Chacon et al. 2006, Peretyazhko and Sposito 2005). This mineral associated organic matter (MAOM) pool is important for long term storage of C and nutrients, believed to remain in the soil from decades to centuries (Trumbore 2009). However, recent research suggests that these pools are more dynamic than previously thought and can exhibit short term changes (Jilling et al. 2020). Under flooded conditions, the soil can become saturated and anoxic (lacking oxygen). This anoxia can mobilize mineral associated C, N and P into soluble and bioavailable C, N and P (Miller et al. 2001) (Fig 1.). Because flood intensity and duration likely influence anoxic conditions, the flood event itself may impact the amount and timing of C, N, and P bioavailability. While increases in soluble and bioavailable C, N and P can benefit ecosystems by increasing crop productivity and microbial growth, relatively higher solubility can also lead to a loss of soil C, N and P through leaching and C mineralization. Whether the newly soluble C, N and P are retained in or lost from the soil during and after flooding will depend in part on interactions with soil minerals and the microbial community.

Soil microbes are important gate keepers of C and nutrient cycling, responsible for much of the transformation and mineralization of organic C, N, and P. Microbes secrete exo-cellular enzymes that degrade complex substrates into accessible forms that can be used for their own growth and incorporated into their biomass (German et al. 2011).

Microbial stoichiometric demand, the specific ratio of C, N and P required by the microbes to grow, may couple C, N and P during and after a flood event. Microbes have a very specific C:N:P demand with fairly inflexible stoichiometric homeostasis, meaning that microbes have little ability to adapt to resource imbalances within the soil (Spohn 2016, Mooshammer et al. 2014). Further, microbes require a relatively high level of P, especially compared to plants, and this demand is thought to be higher during periods of rapid microbial growth (Mori et al. 2017, Mooshammer et al. 2014). When mineral associated C, N and P are released into biologically available forms, microbial access to these frequently limiting substrates increases (Fig 1.). In response to this increased bioavailability of C, N, and P, microbial uptake and accumulation of C, N and P could increase during flooding if microbial activity is maintained during or immediately after flooding, when aerobic conditions return. If microbial uptake increases, these substrates may be temporarily immobilized within the microbial biomass and thus less likely to be lost from the system. (Zhang et al. 2018, Richardson and Simpson 2011). Since microbial C, N and P uptake are linked, an increase in the demand for one will increase demand for the others, essentially coupling the fates of C, N and P (Gross et al. 2020, Zhang et al. 2018, Heuck et al. 2015).

Microbial activity and growth may lead to an overall retention of C, N and P during or after flooding. With higher microbial biomass and activity there is greater potential for C, N and P retention. Further, the forms of C, N and P that are released after microbes lyse are likely to be resorbed into the MAOM pool (Jilling

et al. 2020, Kallenbach et al. 2016). Whether this occurs will depend on the ability of the community to maintain growth during flood conditions or recover immediately after. Lower oxygen concentrations during flooding may reduce microbial growth and activity (Gross et al. 2018). If this is the case, relatively less substrate will be assimilated into microbial biomass or the stoichiometric demands for C, N, and P may shift.

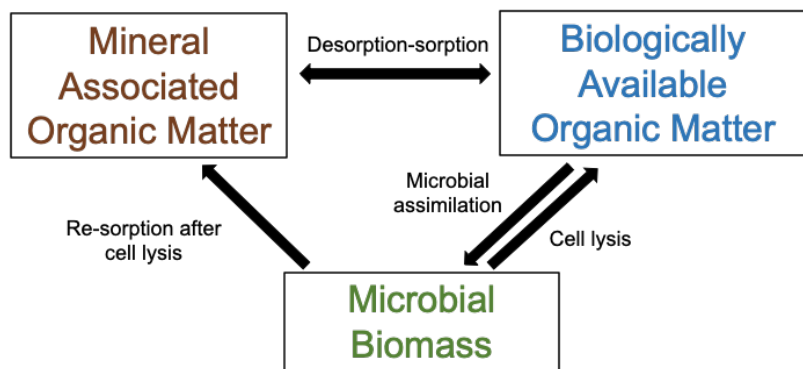


Figure 1. Simplified conceptual diagram of how organic matter is exchanged between the mineral associated organic matter pool, biologically available organic matter pool and microbial community. Flooding may increase the amount of C, N and P that is released from the MAOM pool into the biologically available pool.

We carried out a short-term laboratory incubation to determine how flood conditions: 1) influence the concentrations of C, N, and P in the mineral bound and biologically available pools before, during and post flood event and 2) understand how changes in the mineral bound and biologically available pools impact the microbial community. The controlled conditions allowed us to isolate and understand the impact of flood duration and intensity on C, N and P dynamics.

Soil incubations were maintained at three moisture levels (control, moderate and saturated) to simulate different flood intensities that represent current and projected flood regimes. These moisture treatments were held for three different time periods, representing different flood durations, to understand the interactive effects of flood intensity and duration. We hypothesized that soils held at higher moisture levels with longer flooding periods will show a larger decrease in mineral bound C, N and P and a larger increase in soluble C, N and P. We also anticipated microbial enzyme activity to respond to these flood-induced increases in higher soluble C, N and P.

Methods

Incubation Design

Our experimental design consisted of 3 flood intensity treatments and 3 flood duration periods, replicated 5 times, except for our control (pre-flood conditions) which was replicated 10 times. We also had 3 sampling points, before (control), during and after flooding for a total of 70 sample units. We collected soil from the Emile A. Lods Agronomy research center in Sainte Anne de Bellevue, Quebec in October 2019 from corn fields grown under standard practices of the region at a depth of 0-15 cm. The collected soil was then sieved to 2 mm and homogenized, to ensure that each soil incubation began with a similar soil and microbial composition. We placed approximately 135 g (dry weight) of the homogenized soil in cylindrical columns (10 cm length and 4 cm diameter) with fine mesh (<0.53 μm) attached to the bottom to allow water to drain. To examine flood intensity, we exposed the soil to two different gravimetric water content (GWC) levels representing different flood intensities: 50% (moderate) and 100% (severe). Our control, the pre-flood GWC was 20%, considerably lower than both treatments. The field capacity, a measurement of ideal moisture conditions, for these soils measured at 35% GWC. We kept the severe

treatment soil under standing water after bringing it to 100% GWC, to represent severely waterlogged soil. The moderate treatment represents water logging after a significant rainfall event, common to many clayey Quebec agricultural soils. The severe treatment is indicative of soil water logging that occurs during the seasonal flooding, which is predicted to increase in Northeast Canada.

For our flood duration manipulation, each flood intensity treatment was held for three different flood periods: 0.5 hour, 24 hours and 1 week. These time periods were selected to understand how flood duration affects the retention or loss of C, N and P as well as the interactive effects of flood intensity and duration. Immediately after the designated time period for the duration treatments, a subset of samples was dried by gravity to 20% GWC, to understand soil conditions post flood period, once the soil has dried and the soil has returned pre-flood moisture (Fig. 2). During the incubation period, soil columns were covered with parafilm on top and were kept between 18-20°C in the dark. Leachate was collected after the experimental moisture period and all soils after sampling were kept frozen until analysis.

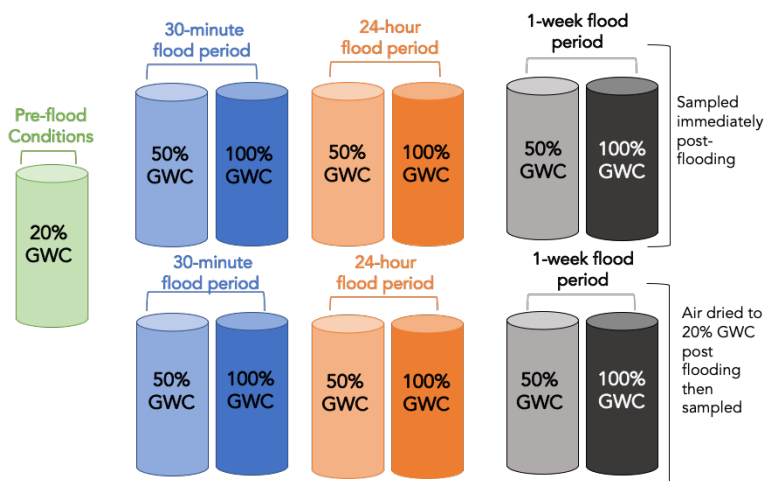


Figure 2. Soil incubations were held at either 50% or 100% GWC. Each GWC was held for three different time periods: 0.5 hour, 24 hours and 1 week representing our flood duration treatments. One set was sampled immediately after the period of moisture manipulation (A) and one set was allowed to return field-capacity 20% GWC (B). Each soil column in figure represents 5 replicates, except for pre-flood conditions which represents 10 replicates (70 total).



Phosphorus Analysis

We used a modified Hedley fractionation to analyze nine P pools that differ in stability (Tiessen and Moir 1993). This report will examine two of these P pools: water extractable inorganic (biologically available) and sodium hydroxide (NaOH) extractable total (mineral associated). The NaOH extracted P pool represents inorganic and organic P that is bound to iron and aluminum oxides. As iron and aluminum oxides are thought to release P during changes in anoxia (Miller et al. 2001), this is the mineral bound pool extracted in the Hedley fractionation most relevant to our study. These extracts were analyzed using malachite green (Ohno and Zibilske 1991).

Carbon and Nitrogen Analysis

We measured water extractable organic C and total N, the most bioavailable C and N pools on a TOC/N analyzer. We extracted mineral bound C and N using 0.5% sodium hexametaphosphate to disperse soil aggregates, followed by sieving to 53 μm to isolate the clay fraction (<53 μm). We then analyzed the isolated mineral fraction on an elemental analyzer (Sokol and Bradford 2019). These pools represent the MAOM pool which is the more stable, and biologically unavailable forms of C and N.

Microbial Analysis

Four exo-cellular enzymes were measured to capture the maximum potential microbial activity in the different flood and post flood treatments, each enzyme corresponds to a different substrate (Saiya-Cork et al. 2002). B-1,4-glucosidase captures the final step of microbial cellulose breakdown to easily assimilate glucose. Acid phosphatase measures the communities' ability to break down organic P. Leucine amino peptidase and tyrosine amino peptidase were used to understand the microbial communities' ability to breakdown N compounds associated with amino acids.

Results and Discussion

How does flood intensity and duration impact mineral bound C, N and P?

The MAOM pool is the largest and considered to be the most stable C, N and P pool, comprising approximately 50-80% of soil organic matter. This pool is thus important for long term soil fertility as well as C sequestration. Small increases or decreases in this pool can dramatically shift soil C, N and P cycles. Recent research shows that this pool can experience short term changes and may be more dynamic than believed, especially under environmental disturbances (Jilling et al. 2018, Keiluweit et al. 2015). Our results show that flooding does indeed induce shifts in the mineral associated C, N and P pools. However, each of these substrates responds differently to the varying flood scenarios, indicating that mineral associated C, N and P are decoupled in their response to this disturbance.

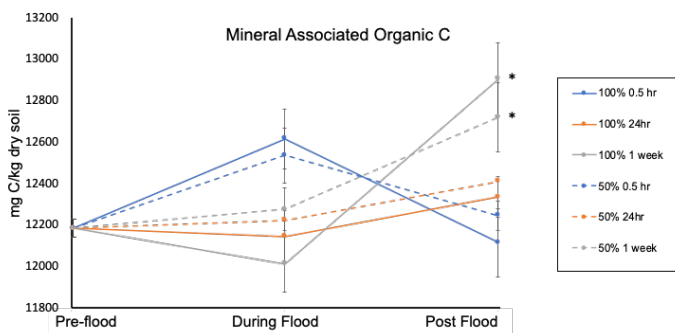


Figure 3. Mean pre, during and post flood mineral bound C concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

Flood duration influenced the magnitude and direction of changes to the mineral associated organic C pool (Fig. 3). There was a surprising increase in mineral associated organic C for both moderate and severe intensity week-long flood treatments post flood. As there were only shifts in the post flood period, these results indicate that much of the transformation of this pool occurs during the resorption, when the soil is drying, rather than in desorption. Flood intensity showed a lesser effect on this pool, influencing the magnitude of the increase of mineral bound C with a greater increase in the severe flood treatment.

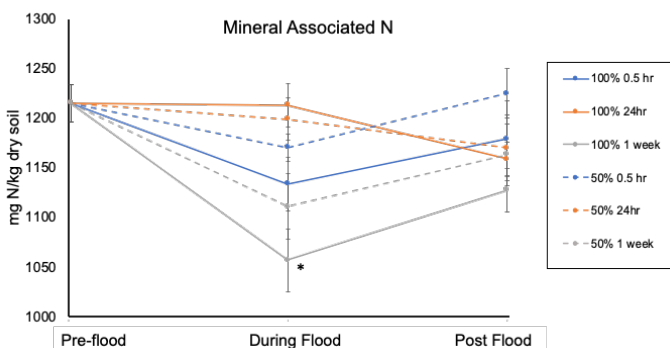


Figure 4. Mean pre, during and post flood mineral bound N concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

In contrast to C, mineral associated N showed relatively small shifts, only decreasing during the flood in the highest duration and severe flood intensity (Fig. 4) This decrease did not remain once the soil dried post flood. Of the three substrates, N seems to be the most resilient to shifts caused by flooding.

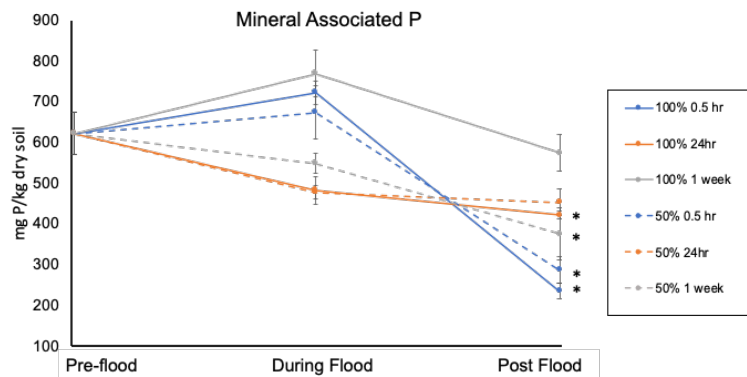


Figure 5. Mean pre, during and post flood mineral bound P concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

The mineral associated (NaOH extracted) P results exhibit more inconsistent changes to flood duration (Fig. 5). Similar to C, flood duration impacted the P pool more than intensity, however each flood duration shows a unique pattern. For the moderate flood intensity both the 0.5 hour and 1-week treatments decreased post flooding. In contrast, in the severe flood treatments there was a significant decrease post flooding for the shorter flood durations (0.5 and 24 hours). These differences indicate that there may be frequent shifts in the mineral associated pool with flooding that were not captured in this study. Further, flood duration and intensity interactively impact how the mineral associated P pool shifts, making it difficult to predict how this pool will change under different flood scenarios.

Our results show that the MAOM pool is susceptible to changes and may even change frequently throughout a flood period. Interestingly, flood duration had a greater influence on changes to the MAOM pool compared to intensity. As flood duration is expected to increase due to climate change, more land may experience changes to the soil mineral bound C, N and P pools (Jeong et al. 2014, Clavet-Gaumont et al. 2012).

Changes to the MAOM pool can have lasting consequences for soil fertility, ecosystem productivity, and the terrestrial C, N and P cycles. Shifts in the mineral associated organic C pool, can also have significant consequences for climate change as soil is an important C sink (Paustian et al. 2016). While we did find an increase in mineral associated organic C post flooding in the 1-week treatments this does not necessarily indicate an increase in stable C. Previous studies showed that increases in mineral bound C are from microbial cells lysing with environmental disturbances (Fierer and Schimel 2002). Additionally, the losses in the mineral (NaOH extracted) P pool may decrease long term soil fertility as this P may be lost from the soil, increasing reliance on P fertilizers in the future.

How does flood intensity and duration impact biologically available C, N and P?

The water extractable organic matter pool represents the soluble and biologically available C, N and P pools. While this pool is significantly smaller than the MAOM pool because of its high rates of production and removal, it is the primary source of C, N and P for the microbial community (Mori et al. 2017, Mooshammer et al. 2014). Thus, changes in soluble organic matter can dramatically impact microbial activity and substrate cycling. Our results show that water extractable C, N and P experience dramatic shifts with flooding. Unlike the MAOM pool, intensity not duration was the determining flood characteristic for changes in this pool though duration impacted the magnitude of the shifts.

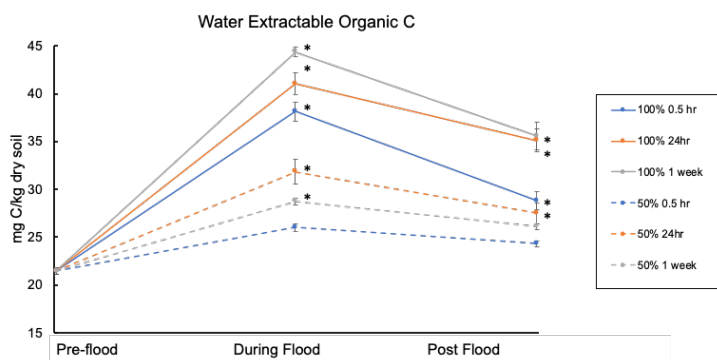


Figure 6. Mean pre, during and post flood water extractable organic C concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

Water extractable C shows an obvious pattern of greater increases in C with greater intensity and greater duration (Fig. 6). All flood durations in the severe flood treatment showed significant increases in the water extractable C during and post flooding. In contrast, for the moderate flood treatment only the two longer flood duration treatments increased during the flood, with only a sustained increase in the 24-hour flood duration.

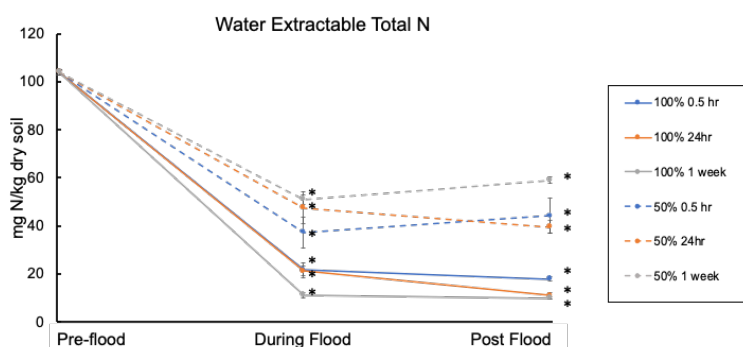


Figure 7. Mean pre, during and post flood water extractable N concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

In contrast to C, water extractable N dramatically decreased with greater flood intensity and duration (Fig. 7). This decrease is largely due to nitrate leaching out of the soil. While, every flood treatment significantly decreased in N, like C, flood intensity influenced the magnitude of the decrease, with the severe flood treatment experiencing greater decreases than the moderate treatment. Also like C, flood duration impacted the degree of N loss, with longer flood periods resulting in greater losses.

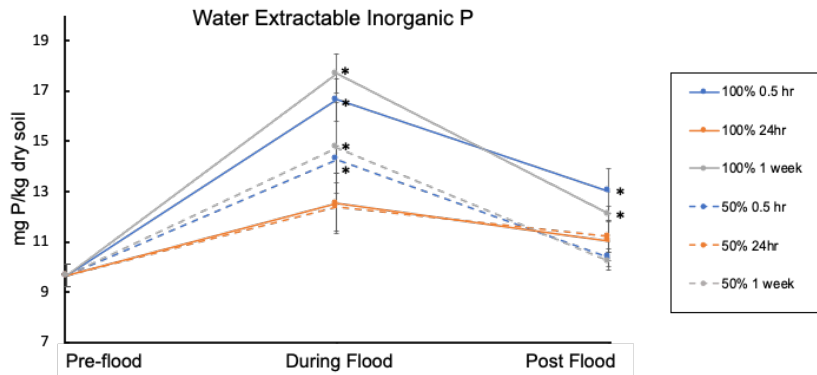


Figure 8. Mean pre, during and post flood water extractable inorganic P concentrations for all flood treatments. Asterisks signify significant differences ($p < 0.05$). Error bars represent standard error of mean ($n = 5$).

Like mineral associated P, water extractable P results indicate a more complicated response to flooding than C and N (Fig. 8). The 0.5-hour and the 1-week flood treatments show a similar pattern to C, with greater flood intensity resulting in greater increases in water extractable P. These increases remain post flood in the severe flood treatment, although at lower levels compared to during the flood. However, the 24-hour flood duration did not shift with flooding. These results, similar to mineral associated P, suggest that the water extractable P pool is experiencing shifts throughout the flood period, which were not fully captured in our study.

The biologically available C, N and P pools experienced the greatest changes with flooding of all measured variables. One important finding was that flood intensity not duration has a greater influence on changes to the biologically available pool. This suggests that even short high intensity floods can dramatically shift the biologically available pool. This is especially relevant given that more land, including farmland, in Northeast Canada is expected to experience high intensity flooding (Jeong et al. 2014, Clavet-Gaumont et al. 2012). While this land may already experience soil saturation due to high rainfall, increases in seasonal flooding which are high intensity, will induce greater shifts in these pools. Even farmland that endures only short periods of seasonal flooding may still experience changes in the biologically available C, N and P pools.

Given the impact that high intensity flooding has on the biologically available organic matter pool, these flood events may greatly impact ecosystem and agricultural productivity. Increases in available P, may temporarily benefit agricultural yield as plants are able to access more of this limiting nutrient. As this increase in P remains post flood, farmers may want to account for this increase in their P fertilizer budget. The increases in C and P in the short term additionally may cause increased microbial activity and nutrient cycling. However, this increase in activity could lead to increases in CO₂ emissions via microbial respiration (Gross et al. 2018). Additionally, as demonstrated by our N results, these available pools are highly susceptible to leaching. This leaching not only decreases soil fertility but may also pollute nearby water sources, degrading water quality (Zhang et al. 2018, Jalili 2009).

How do changes in the biologically available pool impact the microbial community?

Microbes are dependent on C, N and P for their growth and activity (Mooshammer et al. 2014). Thus, increases or decreases in the biologically available pool, like those found in our study will likely change microbial activity. We found that increases in available C and P correlated with increased exo-cellular enzyme activity (Fig. 9). The greater increases in available C and P in the high intensity flood treatments induced high levels of microbial enzyme activity and a stronger correlation. These results show that microbes can maintain and even increase their activity despite the significant disturbance of flooding. In contrast, the large decrease in available N did not prompt decreases in enzyme activity associated with N. This finding suggests that despite the huge losses of available N, microbes are not N limited in this soil.

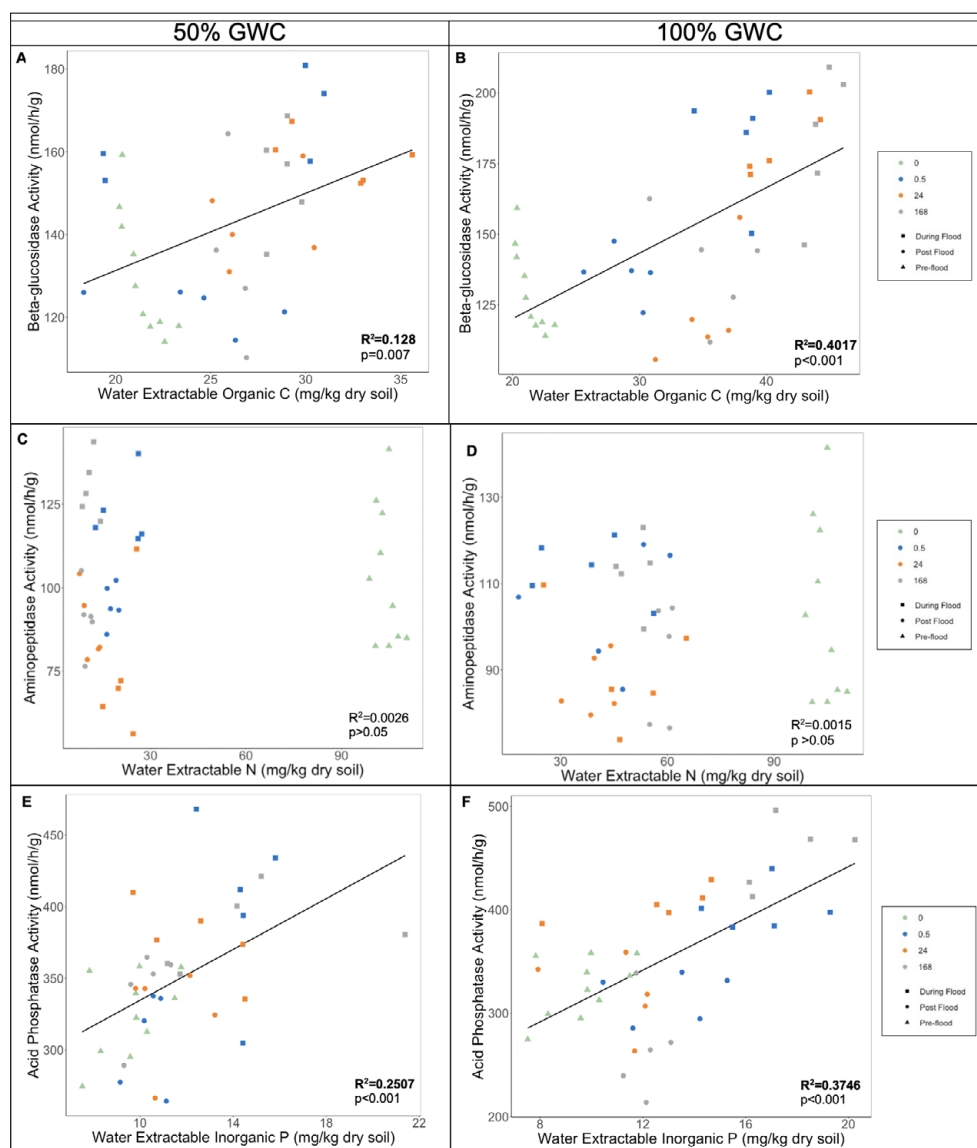


Figure 9. Linear regressions between water extractable C (a-b), N (c-d) and P (e-f) pool and the corresponding exo-cellular enzyme activity. Significance is indicated with bolded R2 values ($p < 0.05$).

The increase in microbial enzyme activity in response to increases in available substrates may help retain C and P in the soil system. Since C and P assimilated into the microbial biomass is retained within the soil matrix (Zhang et al. 2018, Richardson and Simpson 2011). Further, previous studies have shown that microbially processed C and N is more likely to sorb into the MAOM pool after cell lysing (Jilling et al. 2020, Kallenbach et al. 2016). Farming practices that promote microbial activity may further aid this assimilation and retention of newly available C and P.



Conclusion and Policy Suggestions

Our results clearly indicate that both the MAOM and biologically available C, N and P pools are susceptible to substantial and lasting shifts with flooding. Therefore, increases in flooding present a significant threat to Canadian farmland. While farmers and policy makers cannot change the duration or the intensity of a flood event, they can encourage farming practices that build resiliency to flooding.

Agroecosystems are highly managed ecosystems and thus can be managed for C, N and P retention during flood events. Natural Water Retention Measures (NRWMs) are a form of green infrastructure that can help mitigate the impacts of flooding and are particularly popular in Europe (Collentine and Futter 2018). For agricultural land these practices include but are not limited to: buffer strips, cover crops as well as no and low tillage.

Cover crops and buffer strips increase perennial root biomass, which may be a simple way to lessen the effects of flooding. Roots change the soil structure, increasing soil pore space and allowing for greater drainage, which may lower the amount of time soil remains flooded. Additionally, roots may also allow for greater oxygenation of soils, reducing the amount of soil that becomes anoxic and potentially less release of mineral associated C, N and P into the biologically available pool.

Roots also induce microbial activity, with greater root biomass increasing microbial biomass (Helal and Sauerback 1986). Since our results show that microbial enzyme activity increases with available C and P, a healthy and robust microbial community may be able to retain C and P otherwise susceptible

to loss. Utilizing land management practices that incorporate high root biomass may be key for C, N and P retention during flooding and drying periods in agricultural land. Further, since plant diversity increases microbial activity the cover crops and buffer strips should consist of diverse plant species (Lange et al. 2015). Farming practices like planting diverse perennial buffer strips and cover crops that increase root biomass could ensure that the newly available C, N and P remains in the soil.

No and low tillage practices increase water infiltration, preventing waterlogging and flood conditions (de Almeida et al. 2018). Tillage breaks up the natural structure of the soil and can lead to soil compaction, which prevents water infiltration, increasing flood severity. On the other hand, the adoption of no and low tillage practices preserves paths of water flow, like worm middens, and larger soil aggregates preventing waterlogging. As my results show with greater flood intensity and duration there is greater transformation of soil C, N and P. Thus, reducing intensity and duration by increasing water movement through the soil into the groundwater with no and low tillage practices can prevent transformation and loss of soil C, N and P.

With climate change, agroecosystems will face new and pressing threats like flooding. Thus, it is essential to not only build resiliency to current environmental conditions but to prepare for future climatic conditions in Canadian agricultural land. By understanding how flooding impacts C, N and P dynamics farmers can build resiliency into their land before flooding changes the soil environment.

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