Dynamic Soil Depth Differences in Soil Nutrient Cycling in Boreal Podzol Destined for Land Use Conversion and Land Use Intensification

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Chapter 1 Introduction

1.1 Literature Review

Newfoundland currently only produces 11% of the food consumed on the island portion of the province. Recent historic events (Cominelli, 2020) have highlighted the vulnerability of food supply chains and justify the exploration for ways to augment food production capacities. To increase local food production the government of Newfoundland and Labrador (NL) actively supports conversion of natural boreal forest to farmland. Management to enhance the fertility of these newly converted lands commonly involve the application of large amounts of livestock manure or alternately the reliance on the application of mineral fertiliser. Accompanying the growth of agriculture is the expansion of the aquaculture industry. In NL salmon are initially hatched in inland based closed systems resulting in the production of recirculating aquaculture waste solids (RAS) that are rich in N and P. Currently, land based aquaculture facilities are paying for the removal of the organic waste and the material is disposed as septic waste at a cost to the industry. This also foregoes any valorization of the associated nutrients. Environmental regulations do not allow for these wastes to be discharged into the ocean, albeit some permitting for extraordinary circumstances may be possible (*Disposal of Fish, Shellfish and Fish Offal*, 2004). The increase in organic waste from aquaculture and the increase in agriculture activity provides an option for waste utilization. Globally the sourcing of inorganic nutrients from organic waste streams is becoming a priority with the focus on mitigating nutrient losses. Therefore, it is of interest to both divert RAS from the municipal waste management stream and add value by re-using the associated nutrients in food production. Optimal re-utilization of RAS material in NL may lie in agriculture where significant land enhancement will be required to meet sustained

production expectations. Nutrients in waste resources will prove to be essential in limiting inefficiencies and recycling of essential, non-renewable nutrients like phosphorus (van der Wiel et al., 2019).

Local waste streams as nutrient sources

A recent assessment of nutrient content associated with organic waste streams in NL identified that there is sufficient N and P to cover between 50 to 100% of the fertiliser needs for the agriculture activity (Butler et al., 2017). A collaborative effort between the provincial government, Memorial University and Maoi Canada East (Northern Harvest Seafood) identified an opportunity to repurpose the organic waste from land based smolt rearing as a nutrient source in agriculture. In 2018 there were 88 commercial salmon sites producing 15,107 tonnes of organic waste, industry projections expect increases in the excess of 50,000 tonnes annually (Government of Newfoundland and Labrador, 2019)). Given the current expansion of agriculture activity, it is inherent to understand how land use conversion (LUC) affects soil biogeochemistry and thus, the chemical speciation and fluxes of plant essential nutrients (Altdorff et al., 2017; Deng et al., 2014). Specific to conversion events there are changes in the cyclic addition of plant material, removal of organic matter (OM), accelerated mineralization of remaining OM, all affecting the capacity of the soil to act as sink or source especially for carbon (C) and nitrogen (N) (Schulze et al., 2009). Given that much of the soils under boreal forests are acid Podzols, phosphorous (P) is likely to be associated with aluminium (Al) and iron (Fe) phosphates (Sanborn et al., 2011) creating a potential limitation. Liming for pH correction may affect this balance and favour enhanced availability of these P pools while also increasing the potential for formation of calcium phosphates. Tillage, removal of topsoil C, and accelerated microbial activity associated with changes in soil physical

and chemical properties, including any priming effects due to addition of organic or inorganic fertiliser materials, will modify the kinetic behaviour of P (Fuentes et al., 2006; Grand et al., 2014; Mazzilli et al., 2014). There is a need to understand how newly converted and cultivated land respond to the addition of organic and synthetic nutrients.

Across the globe the land-based aquaculture industry is moving towards RAS based systems due to the reduced environmental impacts (Martins et al., 2010) resulting in the need for options for managing the solids in RAS-waste. Effluents from a tilapia rearing RAS culture have been reported to contain an excess of 200 mg/L nitrate nitrogen and a mean total phosphorus of 20-30 mg/L. The greatest risk to fish in the RAS-water is ammonia; increased pH and water temperature (Yeo et al., 2004). Microbial nitrification can oxidate ammonia and thus reduce RAS-water's toxicity but for this the systems must be maintained in an aerobic state. The solids from the RAS (uneaten feed and excrement) are typically removed via filtration and concentrated in waste management settling ponds or basins. The separation of solids may also occur via geotextile bags, belt filters and membrane reactors (van Rijn, 2013). There are currently several ways to dispose of solids from aquaculture RAS systems most are focused on volume reduction (thickening or dewatering) and stabilization. The intent when handling RAS-waste is to dually reduce odor, pathogens and the costs associated with the transport as the water content can exceed 80% of the volume. A more economic and, arguably, environmentally sustainable approach is to employ aerobic or anaerobic lagoons or digestion reactors that can minimize the quantity of the waste by taking advantage of microbial driven mineralization of OM(Adler, 2004; Badiola et al., 2012; Sharrer et al., 2010; Summerfelt et al., 1999).

To avoid environmental contamination land application of aquaculture organic solids should take in account the agronomic requirements of the crop and the environmental capacity to handle application (Bergheim et al., 1993). Another possible benefit of land application is the reduced cost compared to storing the material for later disposal (Yeo et al., 2004). The N and P content of RAS solids could be used to enhance fertility in both established and recently converted agriculture systems. In a greenhouse experiment, an application rate equivalent to 40 t ha^{-1} of RAS waste dry matter (DM) resulted in increased barley yields to 14 t ha^{-1} of DM where a control application of 1000 kg of mineral fertiliser yielded 9 t ha^{-1} of barley DM. A facility with 453,600 kg of swimming inventory can produce enough biosolid effluent to fertilize approximately 40.5 ha of land at the rate afore mentioned (Bergheim et al., 1993). Alternative applications of RAS solids in agriculture have been proposed from research in horticultural sector which evaluated the use of the material as a soilless substrate for vegetable seedling production (Danaher et al., 2016). Organic RAS sludge produced in land-based aquaculture systems can be disposed on land as raw sludge after settling if large land areas are available. There are concerns that without incorporation land application of RAS solids could limit crop development through crust formation; in wet climates an increased risk of nutrient leaching exists raising environmental concerns (Chen et al., 1997). Furthermore, RAS-waste can provide OM to soil, acting as a soil conditioner or a slow-release fertiliser (Adler & Sikora, 2004; van Rijn, 2013; Yeo et al., 2004). To incentivise the land disposal of RAS-waste there is a need to evaluate the performance of RAS-waste in a NL Podzol.

Podzols

Podzols form in wet and cool climates under coniferous forests. Therefore, they are a dominant soils in the circumboreal region (Sanborn et al., 2011; Sauer et al., 2007). Podzols are distributed

across Canada and make up approximate 4% of the earths total land surface. In Canada Podzols are the second most abundant order of soils after Cryosols. In Atlantic Canada the dominant type of Podzol includes the humo-ferric Podzols (Soil Classification Working Group, 1998); in Newfoundland, 55% of the land area soils are classified under the Podzol order, with a total of 44% of the Podzols classified as Ferro Humic Podzols. In its natural state, a typical podzol has a surface organic LFH horizon, due to the accumulation of partially degraded plant material (Orlova et al., 2019; Sanborn et al., 2011). The organic horizon lies above a mineral and clay poor eluvial Ae horizon which transitions to an illuvial B horizon. The Fe, Al and complexed OM enriched B horizon is the criterion for classifying a soil as Podzol. Podzols have low pH and are well to imperfectly drained. Most Podzols in NL are sandy loams and are either humo-ferric or ferro-humic. In Newfoundland, the B horizons typically have less than 40% clay content but still adhere to a minimum Fe plus Al to clay ratio resulting in a reddish-brown B horizon (Sanborn et al., 2011; Soil Classification Working Group, 1998). The low pH from acidic organic litter accelerates the weathering of primary silicates and clay material leading to the release of Al and Fe (Sauer et al., 2007). The distribution of OM in Podzols drives the classification of a Podzol as a ferro-humic, humo-ferric or humic. Podzols store carbon in the B horizons, the second highest organic carbon stock after Histosols (Schulze et al., 2009). Originally it was thought that the OM found in the B layer of the soil was from the leaching of organic acids from the decomposition of the organic layer (Sanborn et al., 2011; Sauer et al., 2007); others have theorized that OM can also come from root tissue (Buurman & Jogmans, 2005). A defining feature of NL Podzols is the presence of a cemented horizon referred to as a placic layer. The placic layer is comprised mostly of high Fe:Al ratio and, distinct to NL, the placic layer can also be associated with Mn. The Fe species found in the placic layer can be goethite, organic complexed Fe and other inorganic Fe compounds. Placic

horizon genesis can be attributed to redox differences in soil profiles resulting in the precipitation of Fe and Mn, there is still uncertainty in the mechanism behind placic layer genesis (Sanborn et al., 2011).

Agricultural Cultivation of Podzols

As indicated by climate change models there will be an increased demand for arable lands in cold climates. An increase in growing degree days along with an increasing demand for food production at local scales would indicate that boreal ecosystems will be favored for conversion to agricultural use (King et al., 2018; Altdorff et al., 2017) developing a broader understanding of how boreal Podzols respond to LUC becomes essential. (Sauer et al., 2007). In Canada the cultivation of Podzols is only common in eastern Canada and in some coastal areas of British Columbia with the majority of Podzols being found in areas unsuitable for agriculture. In Newfoundland, typical agricultural activity on Podzols include small grains, forages and pasture, root vegetables, cabbage, in mixed operations that include dairy, poultry and other livestock. When cultivated, the O, Ae and upper B horizon can be mixed often resulting in the loss of the upper layers. The resulting Ap horizon significantly reflects the chemical properties of a podzolic B horizon raising concerns. Thus, recent converted soils depending on conversion method and management will be more representative of the subsoil B horizon. The agronomic productivity of a Podzol can be limited due to the reduced water holding capacity, relatively low OM, coarse parent material, all leading to fertility unknowns (Sanborn et al., 2011). Climate change models suggest that precipitation will be modified in the future thus affecting soils by impacting runoff, drainage, soil moisture and nutrient kinetics. Current land conversion will also impact the activity of water in soils and in turn modify the carbon and nutrient fluxes found in a natural state. With land modification and changes

in hydrogeological parameters there is an expected impact on leaching of nutrients into watersheds (Altdorff et al., 2017; Sanborn et al., 2011).

Risks of agricultural cultivation in a converted boreal podzol are extensive. For example, erosion from precipitation and high-water infiltration, in the coarser surface horizons increase the risk of nutrients and pesticides leaching into groundwater, especially of soluble nutrients such as N in its mineral forms. Podzols may act as a functional pollutant filter but dependent on the texture and chemistry of the B horizon (Sauer et al., 2007). Accompanying the concerns with LUC is land use intensification. Replacing a natural ecosystem with a less diverse crop profile can increase the demand for nutrients altering biochemical cycles (Allan et al., 2015). Significant amendment to converted lands will need to be applied to help mitigate the limitation of soils that are, low in OM, and sandy in structure and high in cationic metals. The characteristics of boreal forest soils validate concern in a conversion and highlight the need for considerations to restore ecosystem services through beneficial management practices.

Organic matter application Soil organic matter in Podzols

OM in soils is commonly accepted as a quality or health indicator due to the intricate role soil organic matter (SOM) plays in the structure, nutrient cycling and soil ecology (Bünemann et al., 2018; Doran, 1994; Franzluebbers, 2002). In a Podzol OM influences cation exchange capacity in sandy soils and helps buffering capacity against acidification (Buurman &Jogmans, 2005). The eluvial layers of a podzol are comprised of OM derived from coniferous forest, the resulting OM found in these horizons is acidic and only degraded partially. In summary, OM in the mineral horizons of Podzols was traditionally assumed to originate from partially decayed litter from coniferous forest. New theories combine the illuviation of soluble carbon from the forest floor

litter with the turnover of root materials; it is proposed that the decay of root materials drive OM accumulation in well drained boreal systems (Buurman and Jogmans, 2005). The acidity of boreal forest's coniferous litter accelerates the weathering of parent materials and allows for the translocation of Al and Fe from O and A horizons into the B horizon where it interacts with the soluble organic acids also illuviated from the O horizons (Abakumov et al., 2010).

The complexities of OM in podzol B horizons require a greater understanding in terms of sustainable land management. To ensure the sustainable development of Podzols, there is a need to develop an understanding of nutrients like P and N become associated with mineralization and immobilization cycles relative to OM in soil. The energy flow into ecosystems is what best defines the limits and capacity of ecosystems, and govern nutrient cycling (Reiners, 1986). Photosynthesis is affected by environmental conditions such as temperature, water stress and exposure to light. Foremost, the type and intensity of solar radiation influences the light use efficiency of plants. Solar radiation can influence the leaf nitrogen concentration along with photosynthetic capacity (Monteith, 1972). Photosynthesis fixed carbon can be exported to soil via root exchanges and thus influence the nutrient availability and uptake by plants through modifying soil microbial functions. The effect of light is thus impacting entire ecosystems by altering energy and nutrient fluxes. Excessive solar radiation may lower biomass production, under certain circumstances, yet the effects are ecosystem and species specific, accounting for site specific adaptations (Zepp et al., 1998; Gu, 2002). Environmental drivers of SOM mineralization include management history and climatic variables, particularly temperature and precipitation (Schomburgh, 2009): microbiota carrying out mineralization respond to temperature, available moisture, and availability of oxygen. Variable temperature and oxygen levels can increase or decrease catabolism of organic carbon compounds and thus microbial respiration, and subsequently the mineralization of N and P into

plant available forms. Land management such as deforestation will increase the leaching of organic compounds to lower horizons and influence the mobilization of the Al and Fe in the upper horizon. Land managed as grassland after conversion of forest have been found to lower rates of dissolved organic matter (DOM) leaching (Hughes et al., 1990). Due to the naturally low OM in Podzols and the conventional opinions on organic carbon driven soil health, recommendations focus on enhancing SOM contents to support desired productivities. Management techniques that minimize or eliminate tillage are known to improve SOM in the surface horizons, alternatively inversion tillage incorporating crop residues has been show to slow C decomposition and store in deeper layers negating some of the benefits of no till on soil C. It is also possible that no till management may be detrimental to soil C storage at subsurface depth negating the positive accumulation and within confidence intervals possibly creating a net soil C loss in cool temperate sandy soils (Ogle et al., 2019). Addition of organic materials to soils and incorporation may lead to deeper storage of C but also may enhance mineralization. When boreal Podzols are converted from a natural forested state, addition of OM will be required to bring stability and productivity into the newly intensified system. Labile OM addition to soils might trigger a priming effect, which means that the supplementary C instead of increasing total soil C might trigger accelerated microbial degradation of the extant organic soil carbon. For nutrient rich soils a negative priming effect, thus increased C storage, is possible under same labile C organic fertilisation conditions. The direction of the priming is governed by the C:N ratio and the soil microbial community structure. Boreal ecosystems contain 30% of terrestrial carbon (Karhu et al., 2016), and thus there is great value in exploring the dynamics of carbon cycles in boreal podzols under agricultural activity.

Nitrogen and phosphors pools and speciation

In a natural state a forested Podzol has a relatively tight nitrogen cycle where nitrate and ammonia are taken up by plants at rates matching mineralization with very little leaching. When land is converted from a natural state a series of increases in temperature and oxygen results in an increased mineralization rate by an enhanced microbial activity. Nitrogen in a Podzol can be associated with carbon where the largest pools can be found in dead decaying matter. Smaller pools involve dissolved total N which can be associated with dissolved organic carbon found in illuvial layers (Piirainen et. al., 2002). To adequately understand the drivers of the biogeochemical cycles it is important to look at how the mechanisms of mineralization and immobilization are influenced by the soil's parameters. In a Podzol the concentration of organic phosphorus can be assumed to be 4% of the total P where the remaining 96% of the total P may be inorganic (Dalal, 1977). Phosphorus is available to plants in forms of phosphate ions, and its uptake occurs directly through roots and hyphae via mycorrhizal associations. Organic phosphorus can be available to plants after mineralization, usually carried out by soil microorganism (Cade-Menun et al., 2000). Mineralization of organic phosphorus mediates the availability to plants of common soil organic P compounds such as inositol hexaphosphate, lecithin, nucleic acids, nucleotides and glycerophosphate. Overall availability to the plant ultimately relies on the P stores in the soil. If a soil has a low P retention capacity, then the availability of added P would be high. Conversely soils with high P retention capacity limit availability of P. This also affects the availability for P to soil microorganisms that may mineralize organic forms of P. The low availability of organic P to biological transformation may be a result of sorption as well as fixation by soil colloids. Phosphate ions are very reactive and form insoluble complexes with metallic anions including Fe and Al. Nevertheless, under natural conditions P reaches soils mainly in organic forms. The interest in the organic fraction of P in soils is due to the increased mobility compared to inorganic P. The

quantification of organic phosphorus is also important to track the balance of P fixation as inorganic P is assimilated into organic P (by microorganisms), while mineralization of organic P from dead OM and from organic compounds excreted by microorganisms and shed at roots' surfaces (Dalal, 1977). The combination of metallic cations and low pH impact the form of inorganic phosphorus through immobilization (Cade-Menun et al., 2000) making P availability in soils an issue of concern when converting to agricultural land.

Principles of ecology applied to agro-ecosystems

Ecosystem ecology draws on a breadth of disciplines to provide the principles needed to understand the consequences of society's choices. The concept of an ecosystem starts with identifying boundaries. These boundaries can extend to the entire globe, to a certain biological community, such as the boreal forest found in western Newfoundland, or a farmer's field. An ecosystem encompasses all the organisms and abiotic factors. It is also of critical importance to account for human interaction and manipulations of the ecosystem, as a factor that affects both a biotic and abiotic ecosystem elements driving the functional dynamics of an ecosystem. A holistic approach accounts for the complexities of the interactions in even the simplest ecosystem (Stuart Chapin et al., 2012). Ecosystems are open systems where biogeochemical cycles allow matter exchange along flux pathways between various sources (Vitousek &Reiners, 1975). Converting land from a natural state to agricultural use is a form of ecological alteration that creates a new [agro] ecosystem. An idea of eco-efficiency can be applied to best management practices that focuses on producing more value with less impact. To make use of the regulating functions of nature there is a requirement for agroecosystem design to insure continued sustainability, production expectations and environmental conservation (Tittonell, 2014).

In soils the availability of nutrients is contingent on multiple factors. Microbes actively mineralize organically bound nutrients to forms that are readily available to plants. Roots excrete phosphatases that hydrolyze specific fractions of organic P releasing inorganic P. Organic phosphorus mineralization is variably correlated with the mineralization of N and C, with mineralizing activity proportional to the ratios of between C, N, and P of theSOM. The contribution of mineralization in temperate and boreal regions varies seasonally as temperatures vary. Altering the proportion that is left in the soil as organic P compared to C and N when soil is cultivated (Dalal, 1977). The use of elemental ratios can be applied to understand the composition of the SOM in soil: ratios that are higher reflect organic compounds that are active in carbon pools and have not been degraded or transformed to the same degree as lower ratios (Grand & Lavkulich, 2011).

Stoichiometry Applied in Agriculture Science

The scientific method was truly applied to agriculture starting in the 19th century. The publication of the 1st edition of "Organic Chemistry and its Applications to Agriculture and Physiology" 1840 by Justus Von Liebig addresses the factors and conditions governing plants' acquisition of nutrients bringing agriculture into the realms of the scientific method (Russel, 1942; Velho &Velho, 1997). Simultaneous work in England at the Rothamsted agriculture research station occurred under the direction of John Bennet Lawes in partnership with Joseph Henry Gilbert. At Rothamsted continuous long-term experiments were established to evaluate the effects of nutrient and nutrient sources on plant productivity. Long-term experiments have become since then the golden standard for assessment of the impact of management on the factors influencing soil fertility (Poulton, 1996).

The work of Lawes, Gilbert and Liebig questioned the role of nutrient source and application to crop productivity. They also questioned how plants acquire nutrients shaping the basic precepts of plant nutrition and fertilisation research. The works of Liebig and Laws brought the work of Carl Sprengel to the forefront of the ideas which contributed of the Law of the minimum (van der Ploeg et al., 1999; Russel, 1942; Velho & Velho, 1997). During the 1840's Liebig had an ongoing debate with Lawes and Gilbert specifically about the mechanisms by which plants acquire nitrogen. Through lengthy debate the understanding in the emerging field of agriculture as science was that plants acquire nutrients as individual compounds and use them as materials to build their own biomass. This period also sparked the interest in plant uptake and the analysis of tissues to identify what elements where essential for plant growth (Aulie, 1974). The common visualisation of the law of the minimum is a barrel with variable length staves, with the shortest stave (i.e., nutrient but may also be interpreted as other limiting factors such as photosynthetic efficiency) limiting the capacity of the barrel to retain water (Gorban et al., 2011; Harpole et al., 2011). Liebig's summary (Liebig, 1855) of Sprengel's concepts on nutrient availability and limitations (Sprengel, 1828) will eventually become known as [Sprengel-] Liebig's Law of the minimum (van der Ploeg et al., 1999):

1.   "By the deficiency of absence of the one necessary constituent all other being presence, the soil is rendered barren for all those crops to the life which that one constituent is indispensable.

2.   With equal supplies of the atmospheric conditions for the growth of plants, the yields are directly proportional to the mineral nutrients supplied in the manure

3.   In a soil rich in mineral nutrients, the yield of a field cannot be increased by adding more of the same substances"

In time, criticisms of the Law of the minimum mentions that it does not account for the possibility of adaptation, microevolution, ecological succession, or phenotype variability. The paradoxes observed in fact do not refute the law but reinforce that it needs to be interpreted on a gradient (Gorban et al., 2011). In the barrel analogy nutrient pools are treated as static; in agroecosystems these are dynamic. Thus, variations of the law, namely the co-limitation or the multiple limiting hypothesis that have been subsequently proposed are not mutually exclusive and it is reasonable that all theories play a role into how plants respond to their environmental influences (Model et al., 2002; Harpole et al., 2011). After the successive establishment of soil science and agronomy, there was a need for greater understanding and acknowledgment of the agroecosystem. Although many people were instrumental in influencing agricultural and soil science, particular attention must be given to Alfred C. Redfield and William A. Reiners and their contribution to ecological stoichiometry. Much like Liebig, the work of Redfield infiltrated into many areas of natural sciences. Redfield may be credited with a paradigm shift, as Liebig is, by fusing simple concepts of chemistry with ecology to define what is known as the Redfield ratio, which suggests that organisms have optimum ratios of C:N:P in variable conditions. Redfield stated that "In accordance with Liebig's law of the minimum, that constituent of the sea water present in smallest quantity relative to the requirement for growth of organisms will become the limiting factor" (Redfiled, 1958). Reiner's acknowledged that the Redfiled ratio could be applied to create an axiom of stoichiometry stating that organisms have a regularly ordered chemical composition (Sterner & Elsner, 2002). The law of the conservation of matter indicates that organisms must extract items from their environments which then are assimilated into biological tissues. This indicates that elemental ratios can be used to describe not only organisms but the environment in which they exist: "Organic synthesis and thus metabolic rate can be limited by the supply rate of

essential elements" (Rieners, 1985). With the response of the organism of as a result of environment it is important to consider what may be the limitation; as Liebig laid out the law of the minimum, research that followed made clear that complexity of limitations is beyond a single nutrient.

Tilman (1980) proposed a structure where phases may be employed to describe limitations and the limitation could be essential, hemi-essential, complementary, perfectly substitutable, antagonistic or switching, all based on the heterogeneity of the spatial distribution of nutrient resources. Co-limitation can be expanded to include other factors that have an effect on the growth rate beside immediate nutrient availability. For example, co-limitation can impact photosynthetic efficiency or the rate of the decomposition of organic matter as governed by both the substrate availability and the activity of the decomposing microbial community (Tilman, 1980). The application of ratios extend to larger ecosystem scales where elemental composition of net biomass will change as the ratios of leaves to wood decreases during environmental succession providing a measurement of ecological maturity and also used to measure the effect of site remediation on nutrient stoichiometry in plants (Goloran et al., 2015; Vitousek &Rieners, 1975).

In most terrestrial ecosystems plant growth is limited by N and P. Employing the use of elemental ratios can provide insight as plant traits, vegetation composition and species diversity as anthropogenic alterations continually shift equilibriums. Studies dealing with plant nutrition and stoichiometry mostly focus on N and P because they are stated as the most limiting nutrients in crop production (Guignard et al., 2017; Güsewell, 2004; Weih et al., 2016). Plant N: P ratios are of particular use to identify shifts in limitations as they are easily determined and comparable across studies and systems thus strengthening application in varied environments. A major application of nutrient ratios is the ability to measure the impact and response of environmental

change or human intervention though plant tissue compositions. Ratios can be expressed as mass ratio or an atomic ratio, the use of atomic or molar ratios is most common in plant physiological research due to the reflection of actual stoichiometric relationships. Given the conservation of nutrient composition plant tissue the application of stoichiometry in agronomy may assist in fertiliser management modeling yet caution needs to be taken as there is variability associated with different crops and crop storage products (Sadras, 2006). At the least, the use of nutrient ratios can provide insight into the status of a system may it be community of an individual as to what the status of limitation may be on a nutrient status. Insight from mass ratios of N and P can provide reference into what is limiting in the system and ratio of 14 or less indicates N limitation where ratios above 16 represent P limitation and ratios between 14-16 represent what has been described as colimitation where additions of N or P will stimulate a response. It is important to note that the ratio is what identifies limitation not the concentration of single nutrients (Koerselman & Meuleman, 1996).

Conserved traits in elemental stoichiometry

In biological terms, marginal production is the response of primary productivity to the unit change in nutrient availability, so the plant will adjust allocation for expenditures of obtaining each growth resource until it achieves a steady state indicating that growth is eventually equally limited by all resources (Bloom et al., 1985). Thus, marginal production is not linear with increase in resources, and various nutrients might not be equally taken up as growth advances towards maximum. Elemental ratios can thus provide insight into the physiological status of a plant. The relationship between nitrogen and phosphorus expressed as a ratio can be employed to infer the organism's biochemical composition and its physiological state; for example, the critical demand for plants

can be identified as leaf growth demands more nutrients that other tissues and performs photosynthesis which is critical for plant growth. N:P ratios cannot always clearly describe plant physiological state as leaf senescence or luxury consumption can muddle the interpretation of nutrient demand and utilisation (Čapek et al., 2018). The rRNA is the major pool of P in cells and governs protein synthesis rates. Photosynthesis requires Rubisco and other proteins, which incorporate nitrogen carrying amino acids, but that can only be synthesized in the presence of the P rich ribosomes (Ågren, 2004; Tessier & Raynal, 2003). Elemental ratios in living tissue can thus be excellent indicators of physiological states at organismal and cellular levels. Visual characteristics can be related to what happens at an elemental level, and thus visual observation is a valuable part of assessing experimental outcomes (Li et al., 2019).

Plants control resource inequalities by increasing their capacity to acquire what may be the most limiting nutrient. Root to shoot ratios can be indicative of what limits the plant growth. Resource acquisition in excess of what is immediately required, termed luxury uptake, is a response to excessive availability of a nutrient, such as nitrogen or phosphorus, which can be accumulated and stored in plant tissues for delayed metabolism. Plants thus can increase their capacity for resource acquisition in order to maximize growth in an environment where resources vary in time (Bloom et al., 1985; Marklein &Houlton, 2012). The root:shoot ratios of plants are regulated primarily by an adjusted uptake of N and P through signally mechanisms that are sensitive to phloem. Regulation mechanisms in plants essentially increase uptake of deficient nutrient and down regulate the uptake of non-limiting nutrient to maintain appropriate homeostatic relationship between N and P for proper plant function. An example is the storage of P in root tissue as polyphosphate resulting in high P concentrations in situations of high P supply, or of N as nitrate stored in cells' vacuoles. Biomass allocation between tissues (root:shoot ratios are commonly used)

in plants can be indicative of nutrient availability. Roots increase in response to deficiency of both N and P and in limiting condition plants will allocate resources to root tissues which in inverse conditions are increased in nutrient rich conditions (especially N) fostering the growth and development of shoot tissues. The dry matter content of plants may be increased in plants with high N:P ratios possibly reflecting the accumulation of starches amino acids and secondary products. Leaf senescence often is accelerated by nutrient deficiency and in perennial plants P deficiency a high N:P ratio can accelerate senescence more than a low N:P ratio. The growth rate and developmental stage of a plant will determine the ratios based on needs and the availability of given nutrients: young plants will demand N and P based on basic biochemical process associated with photosynthesis, respiration, protein synthesis, and molecular genetic process. Older plants will no longer be as active reducing RNA requirements leading to higher level N:P ratios. N:P ratios are found to correlate positively (Sadras, 2006) with size and thickness of assimilating tissues and negatively with maximal relative growth rate, relative growth rate is proposed to be determined by the amount of ribosomal RNA or the rate of protein synthesis. Due to the high proportion of P concentrated in nucleic acids, low N:P ratios correlate negatively with growth rates and can serve as an indication of physiological response to nutrient availability. One would also expect positive correlations to be found with concentrations of N and P, with relative leaf thickness counter to negative correlation with N:P (Güsewell, 2004). The variations in availability of N and P influence functional traits expressed in plants. N and P are required for photosynthetic processes cell growth metabolism and protein synthesis, a documented constraint that nutrient availability has on cellular processes, (Guignard et al., 2017). The variability in N:P ratios can be associated more closely to the variability in P uptake in crops rather than N. Factors that influence the effect of P variability may not be that P is inherently more dynamic, but that N is more conserved in plant

function and tissue (Sadras, 2006). The stoichiometry of a plant is in part affected by the nutrient availability in the soil, but the ability of the crop to acquire nutrients does not necessarily reflect chemical availability, and vice versa. The growth rate, as affected by other factors than nutrient availability [including growth stage] might drive the demand for nutrients (Weih et al., 2016).

Co-limitation and the multiple limiting hypothesis

The multiple limitation hypothesis states that a plant at optimum fitness, in which the environment dictates, will not be limited by a single nutrient as foraging efficiency for a single nutrient will not be favored over another. Thus, all nutrients or growth factors become limiting thus optimizing growth. Such ideas, like in Bloom et al. (1985) associate resource acquisition with cost and explains that plants will not expend energy to acquire a nutrient if limited by another (Gleesen & Tilman, 1992) The multiple limiting hypothesis offers a situation where both P and N are equally limiting primary production, plants adjust accordingly to the availability of given nutrients, and modify their environments so that N and P are equally limiting when limited the addition of said nutrient will stimulate plant growth (Čapek et al., 2018).

Across ecosystems and time soils regulate nutrient limitation and resource availability. The interactions between plants and the environment are complex making it difficult to predict specific patterns of resource use. Variable soil processes and plant allocations pathways increase the likelihood of colimitation. There are four conceptual patterns around limitation that include: single-resource limitation, i.e. the law of the minimum, classic colimitation, colimitation by trade off and colimitation by substitution (Craine & Jackson, 2010). Conceptually, the idea that nutrients

in autotrophs are co-limited comes from the understanding of how cellular metabolism closely relies on the presence N and P for example.

The interactive roles of N and P suggest a biochemically dependent co-limitation. Given that nutrients are reported in proportions the expectation is that internal concentrations of the limiting nutrient should increase with addition to media while non-limiting nutrients see a proportional decrease (Bracken et al., 2015). A meta-analysis by Bracken et al. (2015) concluded that there is no evidence across freshwater or terrestrial ecosystems that the addition of one nutrient lowered the proportional internal concentration of another. In contrast, studies that looked at single and multi-species experiments, suggest that increased P availability led to both higher P and N internal concentrations in plants. For instance, the addition of P in the form of orthophosphate can stimulate microbial litter decomposition leading to increased N availability to plants. Once again contrasting ideas of single nutrient, the addition of available N was not reported to affect the concentration of P except for marine environments. The lack of interaction indicates the possibility for single nutrient limitation in primary producers, partly driven by differential partitioning among nutrients between available and non-available chemical species. For example, P can exist in chemical species that are not available for biological mineralization thus the addition of N does not influence the availability of P in the same manner as P addition on N.

The absolute availability of nutrients to plants is dependent on the cycling of given nutrients in the environment, including the variable available and unavailable pools of a nutrient. Nutrient cycling and acquisition is thus not to be understood and studied individually; coupling and feedback between nutrient cycles, such as N and P, exist in the environment (Bracken et al 2015). Single nutrient limitation, the multiple forms of colimitation, and the multiple limitation hypotheses all attempt to explain the complex and dynamic relationship in nutrient cycling. The use of ratios in

foliar tissue may be used as sensitive proximate indicators of nutrient limitation to plant growth and also extend as a tool to measure the impact of nutrient addition on ecosystem health (Tessier &, 2003).

Human impact and ecosystems, and ratio interpretations

N:P ratios at an ecosystem scale can indicate impact of changes on functions given the physiological response in which they represent the inclusion of N:P ratios into community and ecosystem models may enhance the predicative impact for change and mitigation (Güsewell, 2004). Nutrient utilization in agriculture has been noted to be affected by management yet the effects of management techniques usually take long term monitoring to confidently quantify impact, there is potential in applying ecological stoichiometry as a tool for early detection of nutrient related effects on crop rotation on crop yields. Although possible, the variance in climatic variables from year to year again solidify the need for long term data collection to help alleviate year to year variability. With this knowledge, plant tissue nutrient stoichiometry can serve as an indicator of climatic changes (Weih et al., 2016)

P nutrient availability

While many elements are essential for plant growth the focus is usually placed on nitrogen and phosphorus given their central role to plant functions. Plants acquire inorganic P directly through roots or via high P affinity arbuscular mycorrhiza (Gul & Whalen, 2016). In situations where N is more available it can stimulate the synthesis of phosphatases (Harpole et al., 2011).When compared to single nutrient addition, the simultaneous addition of N and P to marine freshwater and terrestrial systems increase primary production leading to a conclusion that the demands for

N and P by autotrophs are similar across varied ecosystems (Elser et al., 2007). When stressed due to lack of available nutrients plants re-allocate resources from older tissues to new tissues inducing a reduction in protein and chlorophyll synthesis. This alteration in plant activity will be represented in elemental ratios providing another opportunity for such ratios to be employed for describing the influences of the environment on plant physiological state (Chapin, 1980).

1.2 Overview

The thesis has been prepared in the manuscript style. Chapter 2 focuses on the agronomic performance of tall fescue while chapter 3 explores the theoretical application ecological stoichiometry as a quality indicator for sustainable management. The objectives of the research were to assess how nutrients are cycled in a boreal podzol slated for land use conversion for agricultural practices. A locally relevant forage grass (tall fescue) was tested on two soils taken from the anticipated plow layer of a boreal podzol. The sample site at the time of collection was in the process of land use conversion from a tree plantation to agriculture production. The first chapter gives a compressive overview as to how two soil depths led to distinct availability (i.e., soil fertility parameters) and utilisation by tall fescue of nutrients from mineral or organic fertilisers.

The second chapter employed the theoretical concept of ecological stoichiometry. The application of chemistry to agriculture serves as the foundation for the science of agriculture. When applied to an agricultural setting, the theory of ecological stoichiometry unites agronomy and ecology in a way that helps better understand complex agricultural systems. Macronutrient ratios as indicators, e.g., C:N:P, can be employed to link the kinetic equilibrium between total and available soil nutrients and life sustained by the soil C:N:P. The necessity of C for organism structure and sugars, requirement of N for enzymatically driven anabolism and catabolism, and photosynthesis, along

with the energy currency of P makes the C:N:P ratio an essential indicator. Elemental, molar quantification in plant biomass and soil can serve as indicator on the sustainability of the ecosystem and biotic to abiotic relationship. This provides evidence that, in boreal regions, soil fertility unknowns may be of issue when lands are converted for agriculture. Understanding how best to manage new land is becoming even more important with climate change altering the agricultural production capacities and expectations of boreal soil regions. This work offers a starting point to develop confident recommendations on understanding of how converted podzols will respond to mineral and organic fertilisers as a function of the eventual mixture of soil depths into the newly created plow layer.

Following study chapters:

2) Impact of fertiliser source on the dynamics of carbon and nutrients in a Podzol designated for land-use conversion

3) Impact of soil layers of a converted boreal soil on tall fescue biomass stoichiometry and residual soil nutrients

4) General Conclusion and Recommendations

5) Supplementary Data

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Chapter 2: Impact of fertiliser source on the dynamics of carbon and nutrients in a Podzol designated for land-use conversion

(NOTE: Chapter accepted for publication in the Soil Use and Management journal)

Abstract

Boreal lands have long been considered unsuitable for agriculture. Climate change projections support a shift towards increased agricultural production in boreal ecosystems via land use conversion. Depending on the conversion protocol the new plough layer may variably reflect the properties of different soil depths. This study assessed how adding mineral or organic fertiliser sources to distinct soil layers within ploughing depth (0-15cm [*topsoil*] or 15-30cm [*subsoil*]) affects nutrient uptake and crop productivity. Locally relevant organic waste fertilisers (dairy manure [DMN], recirculating aquaculture system supernatant [SUP] and slurry [RAS]), were compared against mineral fertiliser. Tall fescue was used to verify agronomic responses. Starting available nutrient concentrations were balanced as necessary with mineral N and P fertilisers. Except for NH_4-nitrogen, both soils were of apparent similar fertility (total C <1%); unfertilized, neither could support significant plant growth. *Subsoil*-based growth was fast but limited in time reflecting immediately available nutrients. *Topsoil* led to delayed, extended growth. This might be due to dichotomous priming effects: during the duration of the experiment up to 36% of *subsoil* carbon was lost, while the *topsoil* gained up to 49% carbon, most apparent for DMN. While RAS led to the greatest N and P acquisition efficiencies for *topsoil,* it had no such effect for *subsoil*, suggesting distinct fixation and mineralization processes. Dissimilar C cycling and nutrient acquisition for the two soil depths indicates that site-specific considerations that include soil health parameters to the commonly tested available nutrients are needed where Podzols are farmed after land-use conversion.

1. **Introduction**

Climate change increases the growing degree days in boreal ecosystems (King et al., 2018) prompting considerations of land use conversion (LUC) for agricultural use. If climate change predictions are actualized the current breadbaskets might no longer be able to sustain the demands of the current and future populations. This increases the demand for arable lands in cold climates experiencing climate shifts accelerating northern land use conversion and intensification (LUC/LUI) (Altdorff et al., 2017). The most common soils in the wet and cool circumboreal region are Podzols. In Canada the cultivation of Podzols is only common in eastern Canada and in some coastal areas of British Columbia with the majority being found in areas traditionally considered unsuitable for agriculture. In the province of Newfoundland and Labrador (NL), a region with agriculture fully based on converted boreal lands, 55% of soils are classified as Podzols, of which 44% are Ferro Humic Podzols. Nevertheless, converted Podzols have limited agronomic productivity due to their water holding capacity, relatively low organic matter (OM), coarse parent material, all contributing to fertility unknowns (Sanborn et al., 2011). When cultivated, the O or LFH, Ae and upper B horizon could be mixed often resulting in the loss of the upper layers (Ricketts et al., 2003). However, it is common for the organic surface horizons and the Ae horizon to be removed during conversion and thus lost to variable extents, depending on the land clearing protocol employed. The resulting converted Ap horizon may thus significantly reflect the chemical and physical properties of a podzolic B horizon (Sanborn et al., 2011). One option considered for expanding boreal region agriculture, notable in Eastern Canada, is to also re-purposing formerly abandoned lands. However, non-tilled soils under tree plantations or abandoned agricultural lands, may undergo a partial re-podzolization altering the parameters of the soil layers even before a full podzolic horizonation is evident (Abakumov et al., 2020).

Climate change models suggest that precipitation will be altered in the future thus affecting runoff, drainage, soil moisture and nutrient kinetics. With LUC modification and changes in hydrogeological parameters, the major impact of agriculture will be the risk to watersheds (Altdorff et al., 2017) especially due to soluble nutrients including N and even water-soluble P (Kedir et al., 2021). Replacing a natural boreal forest with an agroecosystem will increase the demand for nutrients, as soil organic matter is depleted, altering biochemical cycles (Allan et al., 2015). LUC changes the input of plant material to soils, accelerates mineralization of remaining soil organic matter due to priming effects driven by tillage and application of organic or mineral fertilisers (Mazzilli et al., 2014), affecting soil microbiological functions. Thus, LUC impacts soils capacity as a sink or source for both carbon and nutrients (Schulze et al., 2009). Specific to Podzols LUC favours phosphorous (P) fixation as aluminium (Al) and iron (Fe) phosphates (Sanborn et al., 2011) increasing the need for fertilization. All these changes modify the nutrient kinetic behaviour (Fuentes et al., 2006; Grand et al., 2014) thus impacting potential agricultural productivity.

Commonly, significant amounts of organic materials are added to soils to enhance the fertility of newly converted boreal lands (Larney & Angers, 2012). A recent assessment of organic waste streams across NL identified that wastes contain sufficient nutrients to cover between 50 to 100% of the N and P fertiliser required at the current intensity of agricultural production (Butler et al., 2017). An organic waste stream that is truly underutilized comes from the use of recirculating aquaculture systems (RASwaste), a common method for smolt production and primarily employed in the northern aquaculture industry. The N and P contents of RASwaste offer a potential fertiliser for both established and recently converted agricultural systems; RASwaste can provide organic matter, acting as a soil conditioner or a slow-release fertiliser and has been reported to increase

barley yields in a greenhouse experiment (Bergheim et al., 1993). There is a need to understand how the soil layers that might be variably mixed during LUC, and thus variably contribute to the new plough layer, respond to the addition of locally relevant fertilisers. This will impact agronomic management and the general capacity of the new farmlands to sustain agriculture. Given the current push to expand northern agriculture, it is essential to understand how LUC affects soil biogeochemistry and thus, the chemical speciation and fluxes of plant essential nutrients (Deng et al., 2014).

The hypothesis for this study is that plant nutrient acquisition will vary with the soil depth of a long-term spruce plantation now designated for tilled agriculture. It is expected that the pre-conversion *topsoil* would favour greater nutrient use efficiency and biomass productivity than the pre-conversion *subsoil*. It is also hypothesized that the type of fertiliser, either mineral or organic, applied at equivalent available N and P amounts, would lead to distinct nutrient uptake rates and crop productivity; these differences would also vary with soil depth due to the state of podzolization.

2. Materials and Methods.

2.1. Soil sampling

Soils for the experiment were collected from a Ferrohumic Podzol, the dominant soil type in the area, at the Centre for Agriculture and Forestry Development, Wooddale, NL, Canada (49. 0246° N, 55. 5498° W, 46 m asl). *Topsoil* (0-15cm) and *subsoil* (15-30 cm) were sampled from 3 locations in the field and mixed to obtain a representative soil, on June 13th, 2018. Soil samples were air dried in the header of a greenhouse, at 20 °C and 40% relative humidity then passed through a 4mm sieve removing large stones and organic debris. The baseline soil measurements

are summarised in Table 1. Historically, the field was a white spruce (*Picea glauca*) forestry plantation established between 1994 and 1996; prior to that there was a native boreal forest. The spruce plantation was regularly fertilized with 250 kg ha^{-1} of 34-0-0 in the summer and 250 kg ha^{-1} of 18-09-18 in the spring and autumn with no use of organic amendments. In October of 2017 the spruce trees were removed, and the soil was worked with a disc harrow at 10 cm and left to rest until the next autumn's deep tillage. At the time of sampling the land had not undergone any ploughing and was destined for potato production.

2.2. Fertilisers and Amendments

Recirculating Aquaculture System supernatant (SUP), slurry (RAS) and dairy manure (DMN) were used as organic fertilisers (Table 1). SUP and RAS were collected at the Northern Harvest Seafood's, Stephensville, NL, Canada. SUP was skimmed from the surface of the settling tank prior to agitation then RAS was collected after thorough mixing. DMN was collected from an open manure pond (Rideout's Dairy, Cormack, NL, Canada) that had been agitated on the sampling day. All organic fertilisers were stored at 4 °C until used. Synthetic fertilisers included ammonium sulphate, $((NH_4)_2SO_4; 21-0-0)$ and triple superphosphate $(CaH_4(PO_4)_2; 0-42-0)$.

Table 1. Soil and organic fertilisers parameters

Initial soil nutrients

Soil	Clay Silt Sand	Bulk Dens. (g cm⁻³)	Soil Moist. (%)	NH₄-N NO₃-N	TC OC IC	Extract. P	Extract. K	pH	Buffer pH
	%			(% dry soil)					
Topsoil	10.16 12.19 77.65	1.4	17.3	3.65×10⁻⁰⁴ 6.26×10⁻⁰³	0.96 0.93 0.02	1.79×10⁻⁰³	1.27	5.3	6.4
Subsoil	10.20 12.24 77.56	1.4	16.1	3.31×10⁻⁰⁵ 3.59×10⁻⁰³	0.85 0.85 0.02	1.43×10⁻⁰³	0.94	5.3	6.4

Organic Nutrient Sources

Nutrient	Dry Mat. (%)	TKN†	NH₄-N NO₃-N	TC OC IC	Extract. P	Extract. K	C:N ratio‡
		(% Dry Matter)					
DMN	1.79	7.26	4.79 0.02	34.6 5.01 29.6	1.12	8.66	4.8
RAS	17.7	6.27	1.33 0.0009	27.2 0.93 26.3	9.38	0.04	4.3
SUP	0.05	16.00	4.22 0.15	NR NR NR	2.00	~0	1.7§

† TKN, total Kjeldahl nitrogen
‡ C:N ratio, TC:(TKN+NO₃-N); TKN includes organic and NH₄-N
§ TC% was assumed to be similar to RAS (NOTE: RAS is obtained by concentrating SUP solids)

2.3 Experimental design

Pot experiments were carried out in a greenhouse at the Centre for Agriculture and Forestry Development in Wooddale NL from August 8th, 2018 to October 30th, 2018. Temperature was maintained between a nightly minimum of 18°C and a daily maximum of 25°C with 40-50% relative humidity levels. Natural light, employed throughout the experimental period, averaged at 12.6 h d^{-1}, with 14.76 h d^{-1} at the start of the experiment and 9.97 h d^{-1} at its conclusion. There were 10 treatments (2 soils x 5 fertiliser types [4 fertiliser plus negative control]) each with 5 replicates placed in a randomized block design (Table 3). Pots had a 15 cm diameter and 15 cm depth (equivalent to the average ploughing depth), thus holding 2.65 L of soil. The random number sequence generator in Microsoft Excel was used to assign pots in 5 rows, each row representing one experimental block. Each block contained 1 replicate of each treatment for a total of 50 runs. Tall fescue (*Festuca arundinacea, cv* Kokanee;) was seeded by hand via surface broadcasts. In the first 14 days after establishment germination counts where performed on weekly increments, no thinning was performed, and germination was found to be uniform amongst treatments at an average of 22 plants per pot. Fertiliser rates were calculated to provide the same apparent available nutrient amounts (Table 2). Calculations for the organic fertilisers were based on the measured available nitrogen (N) and total phosphorus (P) concentrations. The N and P requirements were based on established agronomic recommendations for tall fescue: 200 kg N ha^{-1} and P requirement of 110 kg P ha^{-1} (Moran et al., 2017). Calculations for organic sources were carried so that the application rate would not exceed neither N, nor P, or the soil water holding capacity. To ensure equal availability organic fertiliser treatments were partially amended, as required, with appropriate mineral fertiliser. K was not considered as it was in sufficient supply in the soil (Table 1). To ensure homogeneity across replicates soil and fertilisers for each treatment were mixed in

batches, concomitantly for all 5 replicates. An amount of 2.65 L of air-dried soil and 440 ml of liquid was used per pot bringing treatments to an initial 80% soil field capacity for water (Table 2). Pots were watered three times weekly; pots were weighed and supplemented based on losses. On average each pot received 231 ml of water at each watering resulting in a sustained soil moisture at 80% of field capacity. The treatments receiving synthetic fertiliser only were considered as positive controls. Treatments without any fertiliser or amendment were employed as negative controls. After harvest soil was tested for total carbon (TC), total N (TN), ammonium and nitrate (available N), extractable P (ExP), extractable magnesium (ExMg) and extractable potassium (ExK) and pH.

Table 2. Experimental treatments

Soil	Treatments[†]	Organic fertiliser (g DM per pot[##])	Mineral fertiliser (g per pot)	
			$(NH_4)_2SO_4$ (21-0-0)	$CaH_4(PO_4)_2$ (0-42-0)
Topsoil (T)	Control (soil only) (T_{NEG})	na	na	na
	Mineral fertiliser (T_{POS})	na	0. 976	0. 457
	Dairy manure (T_{DMN})	4. 74	na	0. 252
	RAS (T_{RAS})	14. 34	0. 907	na
	Supernatant (T_{SUP})	0. 02	0. 533	0. 454
Subsoil (S)	Control (soil only) (S_{NEG})	na	na	na
	Mineral fertiliser (S_{POS})	na	1. 488	0. 538
	Dairy manure (S_{DMN})	6. 45	na	0. 345
	RAS (S_{RAS})	22. 47	1. 423	na
	Supernatant (S_{SUP})	0. 02	1. 045	0. 453

[†] Calculated field water holding capacity was of 0. 175 L per L of soil;
[‡] Each pot had a volume of 2. 65L; repacked at a bulk density (BD) of 1. 2 kg L[-]

Table 3. Graphical representation of experimental set up, experiment was set up in randomized block design atop greenhouse bench, numbers above columns represent blocks, treatment codes available in table 2. Experimental treatments

1	2	3	4	5
T_{RAS}	S_{RAS}	S_{POS}	T_{SUP}	T_{NEG}
S_{POS}	T_{SUP}	T_{SUP}	S_{SUP}	T_{POS}
S_{NEG}	T_{NEG}	S_{DMN}	S_{DMN}	T_{SUP}
T_{DMN}	S_{DMN}	T_{NEG}	S_{POS}	S_{NEG}
S_{SUP}	S_{NEG}	S_{SUP}	T_{NEG}	T_{DMN}
T_{SUP}	T_{DMN}	T_{DMN}	S_{POS}	S_{POS}
T_{NEG}	T_{POS}	T_{RAS}	T_{DMN}	S_{RAS}
T_{POS}	S_{SUP}	S_{NEG}	S_{NEG}	T_{RAS}
S_{RAS}	S_{POS}	S_{RAS}	T_{RAS}	S_{DMN}
S_{DMN}	T_{RAS}	T_{POS}	S_{RAS}	T_{NEG}

2.4. Plant parameters

Biometrics: leaf stages, and plant vigour assessments were done weekly (data not reported). Plant heights were taken from 5 randomly selected plants in each pot and recorded. Soil Plant Analysis Development chlorophyll (SPAD) measurements were collected on September 12th, September 25th, October 9th and October 29th of 2018 [days 35, 48, 62 and 82] using a Konica Minolta SPAD-502 chlorophyll meter (Coste et al., 2010), three readings were taken per pot.

Plant growth: Growth was described as the cumulative plant length above soil (cm), based on weekly measurements of the maximum length from the soil surface to the most distal leaf tip. Absolute growth rate (AGR, *eq.1*) between measurements was calculated where H_n is plant height at measurement time, H_{n-1} is the plant height at the previous measurement, t_n is time at measurement, t_{n-1} time of the previous measurement (t in days). As AGR reached a maximum at day 42, values were calculated for this interval and total, cumulative growth rate (TGR, *eq.2*) was calculated separately for the total length of the experiment 84 days (Table 4). TGR was calculated where H_n is plant height at measurement time, divided by t_n time at measurement

$$AGR = [H_n - H_{n-1}] / [t_n - t_{n-1}] \qquad eq.1$$
$$TGR = H_n / t_n \qquad eq.2$$

2.5 Plant and soil sampling and analysis

N and P acquisition efficiency (*eq. 3*) were calculated as their proportion in plants RS (roots and shoots) versus the initially available N and P applied to the treatment applied as a combination of mineral and organic fertilisers (Table 2). For N in soil the sum of NH_4-N and NO_4-N was used; for P the soil available P and added fertiliser P were summed.

$$Nutrient\ aquisition\ efficiency\ \% = \frac{intial\ available\ amount\ per\ pot\ (mg)}{plant\ tissue\ total\ per\ pot\ (mg)} \qquad eq.\ 3$$

The aboveground (shoots) and belowground biomass were harvested separately from each pot. Control treatments T_{NEG} and S_{NEG} were harvested on October 5th, 2018, as they stopped growth noted from no difference in height from days 35 (September 19th) to 52 (October 3rd). All the other treatments were harvested on October 29th 2018. At harvest a final plant height measurement was taken (5 measurements per pot); leaf blade width for 3 leaves per pot were recorded along with SPAD measurements. Plant shoots were clipped at approximately 1 cm above the soil surface and fresh biomass weighed in a paper bag. Each pot was then turned over and tipped into a plastic mixing tub. The roots had become bound holding all soil together. The soil and roots were then manually separated allowing for collection of soil and root tissue. Three root length measurements were recorded per pot. Separated roots were rinsed with tap water to wash off bound soil particles; roots were then blotted with paper towels and allowed to dry in paper bags overnight before recording the fresh weights. Roots and shoots where then dried at 60 °C for 48 hours. Root separated soil samples were mixed thoroughly and 50 ml aliquots were stored in capped tubes at -20 °C. The remainder of the soil was split into three equal subsamples, labelled, and randomly allocated for nutrient testing, P sorption study, and storage. Soil samples for nutrient testing where air dried at 22 °C with a relative humidity of 40%. Dry soils were passed through a 2 mm sieve and two 80 mL aliquots were stored at 4 °C and -20 °C, respectively, until analysed. Soil and organic fertiliser chemical testing were performed at the Agriculture and Food Laboratory, University of Guelph Laboratory Services. Soil texture was analysed via the hydrometer method (Bouyoucos, 1962; Kroetsch and Wang, 2007). Soil field capacity for water was calculated via the Saxton method (Saxton, 2006). Soil bulk density was determined by the laboratory as the sieved soil density, due to the loose nature of the collected field sample. pH was read on a water saturated

paste. Soil cation exchange capacity was calculated from the K, Mg, Ca and Na extractable with the Ammonium Acetate method and quantified on an ICP-OES (Simard, 1993).

Soil and organic fertiliser available P was extracted with the Olsen method, standard procedure at the laboratory, and quantified colorimetrically (Reid, 1998). Total carbon and/or TN contents in soil, plant, and organic fertilisers were measured with the Elemental Vario Macro Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany). Inorganic carbon was then calculated by subtracting OC from TC. Soil carbon changes were calculated where soil carbon [sTC] plus the added organic carbon [AOC] were subtracted from the total soil carbon [FSC] at the conclusion of the experiment (*eq. 4*).

$$Change\ in\ C = FSC - (sTC + OC) \qquad\qquad eq.\ 4$$

2.5. Data management and statistical analyses

For the statistical analyses, the R-project software package Version 3. 4. 0 (R Foundation for Statistical Computing) was used; trends in data were explored using general linear modelling. Minitab13 was employed for one way ANOVA and Tukey post hoc analysis (Minitab LLC., 2021). PAST3 was used for the linear discriminant analysis (LDA, Hammer et al., 2001). Data for LDA was standardized as z-scores prior to analysis. The Anderson-Darling test for normality was employed.

3. Results and Discussion

3.1. Mineral and Organic Fertilization effects on Growth rate

An ideal situation for sustainable farming on Podzols would involve the use of organic nutrients. Our results showed that unfertilized soils could not sustain any significant growth, a sign of nutritional deficiency therefore confirming the need for fertility enhancement upon conversion. The growth of tall fescue was impacted more by the soil depth than by nutrient sources. For the *topsoil* organic fertilisers led to growth rates (i.e., AGR) larger than the positive control; the reverse was true for the *subsoil*. Nevertheless, this result should not be employed to suggest the absolute utility of RAS for boreal podzols, as at conversion the topsoil would be mixed with the subsoil. The *subsoil* treatments, including the positive control (S_{POS}), led to faster growth than the *topsoil* (i.e., slopes >1 for the AGR linear fits between *subsoil* treatments and T_{POS}) apart from T_{RAS} (i.e., slope >1 for the AGR linear fits between T_{RAS} and S_{POS}) (Table 4). On the other hand, barring DMN, the *subsoil* led to slower growth (AGR) when amended with organic fertilisers versus mineral fertilisers (i.e., vs S_{POS}).

For the *topsoil*, by the conclusion of the experiment the TGR (i.e., average growth rate for the entire experimental period) for the DMN and RAS amended soils, but not for SUP, has surpassed that of the positive control. This sustained growth rate compared to mineral fertilization would suggest a degree of conservation of initial applied nutrients that could be stored in soil carbon pools representing microbial communities (Wild et al., 2017). For the *subsoil* the TGR was only marginally greater than the positive control (S_{POS}) for DMN, but not for SUP and RAS, suggesting a generally slower overall growth with organic fertilisers than with mineral fertiliser.

These results indicates that the *subsoil* treatments had a more limited capacity to utilise organic fertiliser supplied nutrients, both initially and later in the experiment, while the *topsoil* treatments

had only an initial limitation to utilise the organic fertiliser supplied nutrients (i.e., by day 42); this limitation was eventually overcome (i.e., by day 84).

Thus, the rapid initial growth on *subsoil* was likely driven by the immediate availability of nutrients. The delayed growth on *topsoil*, was a likely response to initial retention of available nutrients in the soil biomass followed by a delayed release of nutrients, i.e., mineralization of organic matter (Eghball et al., 2002). As mineral nutrients were eventually exhausted, continuous mineralization of OM from applied waste material is expected to continue (de Ruiter et al., 1993). It might be hypothesized that the *topsoil* may have supported a more functional microbial community that influences the flow of energy and nutrients to both plant and community decomposers (Wardle et al., 2004). According to this argument the *subsoil* differed in microbial functional state, and probably in abundance and community diversity (Fritze et al., 2000), which affected the sustained growth rates past the apex of the AGR, an opposite response to the one for the *topsoil*. It may be assumed that the way initial available nutrients were provided to the *subsoil,* impacted the potential for resource exhaustion, a condition not reported for the *topsoil* (Fuentes et al., 2006). Other explanations include the chemical nature of podzols; as soil depths increase Al, Fe and clay concentrations increase which can then limit the availability of applied organic carbon from the organic nutrient sources (Grand et al., 2014). These results raise interesting questions regarding the functionality of podzols across depths as it affects nutrient availability, to be considered in more targeted assessments.

Table 4. Average absolute growth rate (AGR average) and total cumulative growth rates (TGR): Linear comparison of organic fertilization to mineral control in two boreal podzol top (0-15cm) and sub (15-30cm) soils

Treatments	AGR average [†] (42 days) (cm d^{-1})		TGR (84 days) (cm d^{-1})	
	Fitted equation	r^2		r^2
Topsoil Growth rates				
Linear Fit versus T$_{POS}$				
T$_{NEG}$	y = 0. 452x + 3. 8424	0. 91	y = 0. 3652x + 4. 9849	0. 89
T$_{DMN}$	y = 1. 0816x - 1. 6668	0. 99	y = 1. 0296x - 0. 9115	0. 98
T$_{RAS}$	y = 1. 1979x + 0. 6405	0. 98	y = 1. 0787x + 2. 2083	0. 98
T$_{SUP}$	y = 1. 068x + 1. 3894	0. 98	y = 0. 9765x + 2. 638	0. 97
Linear Fit versus S$_{POS}$				
T$_{NEG}$	y = 0. 3843x+4. 0419	0. 88	y = 0. 3625x + 3. 8632	0. 94
T$_{POS}$	y = 0. 8347x - 0. 3056	0. 98	y= 0. 95x - 1. 8637	0. 96
T$_{DMN}$	y = 0. 9064x - 2. 0766	0. 98	y = 0. 9907x - 3. 1841	0. 97
T$_{RAS}$	y = 1. 0188x - 0. 1474	0. 99	y = 1. 0533x - 0. 6074	0. 99
T$_{SUP}$	y = 0. 9091x + 0. 6699	0. 99	y = 0. 9552x + 0. 0417	0. 94
Subsoil growth rates				
Linear Fit versus S$_{POS}$				
S$_{NEG}$	y = 0. 3843x + 4. 0419	0. 88	y = 0. 3316x + 4. 761	0. 87
S$_{DMN}$	y = 0. 9735x - 0. 4198	0. 99	y = 1. 0067x - 0. 8852	0. 99
S$_{RAS}$	y = 0. 946x + 0. 7495	0. 96	y = 0. 9035x + 1. 3244	0. 97
S$_{SUP}$	y = 0. 9853x + 0. 6636	0. 98	y = 0. 9416x + 1. 2692	0. 98
Linear Fit versus T$_{POS}$				
S$_{NEG}$	y = 0. 4399x + 4. 5589	0. 81	y = 0. 3213x + 6. 1044	0. 76
S$_{POS}$	y = 1. 1769x + 0. 7544	0. 98	y = 1. 0148x + 2. 90	0. 96
S$_{DMN}$	y = 1. 1608x + 0. 037	0. 99	y = 1. 0067x - 0. 8852	0. 97
S$_{RAS}$	y = 1. 1336x + 1. 0898	0. 97	y = 0. 9035x + 1. 3244	0. 96
S$_{SUP}$	y = 1. 1682x + 1. 2478	0. 97	y = 0. 9416x + 1. 2692	0. 97

[†] AGR average, defined as the average of the growth rates measured between measurements dates

3.2. Plant yields and growth response

The biomass yields were related to the plant heights and the SPAD values (Table 5). Shoot yields were significantly positively correlated ($p < 0.001$) with SPAD values ($r = 0.81$), plant height ($r = 0.82$) and leaf width ($r = 0.81$). As also reflected in TGR (Table 4), a larger yield was measured for all *subsoil* fertilized treatments compared to *topsoil* treatments, with the notable exception of S_{RAS} to T_{RAS}; the factorial interaction between nutrient and soil had a significant negative impact on biomass ($p = <0.001$, coeff. -11.36). Except for the negative controls, the narrowest leaves were measured for S_{RAS}, another confirmation of the limited impact of RAS applied to *subsoil*. All measured plant growth parameters offered a similar response to the impact of the treatments on plant productivity. Under sufficient and balanced nutrient availability increased metabolic activity results in increased production of functional and structural proteins, cellulosic and lignin macromolecules, which allows for larger plant organs (Ågren, 2004). Sufficient nutritional supply was clearly suggested by the measured leaf blade width at harvest. Larger leaf surface area enhances total photosynthesis, further accelerating growth and biomass accumulation (Errecart et al., 2012).

In the field the differences in root mass and penetration depth might have varied with distinct nutrient availability profiles (Henneron et al., 2020). For this pot experiment, with little difference and no statistical significance in total root mass and length, there is no evidence that the root length or biomass had influenced how rhizosphere nutrient availability may have been affected in this experiment. Nevertheless, root length was reduced in the *subsoil* mineral control (S_{POS}). Plants in S_{POS} had to commit fewer resources to root tissue, further evidence that the mineral fertiliser added to the *subsoil* was immediately available to plants, likely with minimal intervention of any soil functions, biological or otherwise (López-Bucio et al., 2003).

Table 5. Plant yield and growth parameters

Treatment	Dry matter yield (Mg ha^{-1})			Chlorophyll (SPAD units)	Height (cm)	Root Length	Leaf width (mm)
	Shoot	Root	Shoot:Root ratio				
T_{NEG}	1. 53 d	2. 26 a	0. 69 a	2. 53 d	18. 64 c	21. 33 a	1. 00 e
T_{POS}	3. 28 c	3. 57 a	1. 02 a	29. 33 ab	34. 96 b	21. 80 a	3. 47 b
T_{DMN}	4. 87 b	8. 72 a	0. 86 a	27. 86 b	35. 52 ab	22. 33 a	3. 60 b
T_{RAS}	7. 13 a	6. 28 a	1. 52 a	36. 26 a	38. 80 a	22. 53 a	4. 93 a
T_{SUP}	4. 81 b	4. 64 a	1. 20 a	20. 37 c	34. 76 b	21. 60 a	2. 80 cde
S_{NEG}	0. 96 d	1. 53 a	0. 72a	1. 35 d	16. 36 c	23. 53 a	1. 00 e
S_{POS}	4. 70 bc	4. 87 a	1. 20 a	30. 42 ab	36. 16 ab	17. 67 a	3. 33 bc
S_{DMN}	5. 66 b	7. 42 a	1. 08 a	23. 44 bc	35. 28 ab	20. 93 a	3. 46 b
S_{RAS}	4. 70 bc	5. 32 a	1. 40 a	26. 71 bc	33. 00 b	21. 27 a	1. 86 de
S_{SUP}	5. 10 b	4. 08 a	1. 46 a	25. 99 bc	35. 48 ab	22. 87 a	2. 86 bcd

In each column means followed by same letter indicate that they are statistically similar based on Tukey's test for pairwise mean comparison

3.1 Soil Carbon, Nitrogen and Phosphorus dynamics

The acquisition profile of the nutrients from all fertilized *subsoil* treatments, suggests that despite evidence of OM mineralization the organic fertilisers applied to the *subsoil* were not a net source of nutrients, as it was the case for the *topsoil*. T_{RAS} best supported plant growth in conjunction with the largest N and P acquisition efficiency (Table 6), suggesting that RAS led to sustained nutrient availability in the *topsoil*. The larger than 100% acquisition of available N in T_{RAS} combined with an increase in soil's C content (Table 6) would suggest a likelihood for mineralization of N in soil. The likelihood for biological N fixation (BNF) was not assessed yet a better functioning soil might more likely allow for BNF, in the context of the available N gradients (Vance, 2001). Furthermore, the *topsoil* sustained active growth for longer (Table 4) while also providing a more sustainable use and conservation of nutrients applied as seen in the plant response and nutrient acquisition, a result that if found in field setting would confirm common recommendations for the conservation of upper soil layers in land use conversion and intensification events.

Table 6. Soil C, N and P at harvest and N and P acquisition efficiency.

Treatment	Total C	Change in Total C	Total N	available N [†]	Olsen P	Acquisition Efficiency (% of initially available forms)	
	% dry soil		mg /100g soil dry			N	P
T_{NEG}	0. 94 c	-0. 01 c,d	72 c,d	0. 18 b	3. 29 d	68 c	45 a,b
T_{POS}	1. 22 b	0. 26 a,b	92 a,b	0. 99 a,b	4. 46 b	55 c	17c
T_{DMN}	1. 49 a	0. 47 a	98 a,b	0. 18 b	4. 51 b	87 c	31 b,c
T_{RAS}	1. 22 b	0. 11 b,c	102 a	2. 14 a	7. 22 a	164 b	58 a
T_{SUP}	1. 21b	0. 25 a,b	86 b,c	0. 21 b	4. 78 b	56 c	21 c
S_{NEG}	0. 62 d	-0. 22 d,e	54 e	0. 09 b	2. 42 e	244 a	29 b,c
S_{POS}	0. 69 d	-0. 15 d,e	64 d,e	0. 58 a,b	3. 19 d	78 c	24 b,c
S_{DMN}	0. 72 c,d	-0. 20 d,e	68 d,e	0. 03 b	2. 71 e	78 c	20 c
S_{RAS}	0. 64 d	-0. 31 e	64 d,e	0. 78 a,b	2. 76 e	77 c	17 c
S_{SUP}	0. 68 d	-0. 16 d,e	68 d,e	0. 05 b	3. 96 c	71 c	21 c

[†] Sum of the NH4-N and NO3-N at harvest
In each column means followed by same letter indicate that they are statistically similar based on Tukey's test for pairwise mean comparison

Figure 2. Plant responses to soil and fertilizer types (Linear discriminant analysis, LDA).

3.2 Role of soil carbon

Available nutrients can be added with fertilisers or be provided through the mineralization of soil organic matter. Respiratory losses of carbon, an indicator of C mineralization, might be employed to describe availability of mineralizable soil N (Schomberg et al., 2009). Addition of small amounts of low molecular weight C substrates, i.e., the more easily available C pools, could elicit signals that lead to an increased microbial activity (Blagodatskaya & Kuzyakov, 2008) triggering a priming effect. Depending on the status of the soil microbial community either positive or negative priming might occur. Accumulation of C in soils may occur when the added organic substrates have large molecular weight C compounds that associate with soil mineral surfaces and humify (Ohno et al., 2007). This accumulation of soil C can be considered a negative priming effect, triggered by the addition of easily available organic matter to soils with small or dormant microbial communities (Perveen et al, 2019) a result found for the *topsoil* treatments (Table 6). Accumulation of OM in soil is assumed to respond better to organic application rather than mineral fertilisers alone, even when equal amounts of potentially plant available N and P are applied (Maillard & Angers, 2014) a result akin to this pot study. However, *topsoil* that received largely easily mineralizable organic waste (e.g., SUP and RAS) or even mineral fertilisers did also accumulate C, an indication of a stable soil microbial community that likely employed N to fix C. As the ideal C:N ratios of 8:1 for bacteria, and 15:1 for fungi (Zhang & Elser, 2017) are above the C:N ratios of the added fertilisers (Table 1), it is likely that some of the fixed C was delivered by plant roots or from the SOC pool to an active microbial community. For the *topsoil* control (T_{NEG}) the soil carbon content did not change during the experiment (Table 5), although the yields (Table 5) were larger than for the control *subsoil* (S_{NEG}) which lost carbon. Moreover, the N acquisition efficiency for the unamended control soils, described as the proportion of initially available N

recovered as total plant N (Congreves et al., 2021), was 68% for *topsoil* (T_{NEG}) but 244% for *subsoil* (S_{NEG}). This offers a possible explanation that the N taken up from *subsoil* was possibly also supplemented through mineralization of SOC (Schomberg et al., 2009). These observations might suggest that addition of available N to the *topsoil* led to both mineralization and C fixation, with an eventual net positive C accumulation. S_{NEG} lost 22% SOC suggesting that once cropped and managed, its C was mineralized, releasing its constituent nutrient ions which contributed to plant uptake. Furthermore, the same *subsoil* had C losses for all fertilisers, statistically equivalent for all treatments, but with the largest losses for S_{RAS}. The NH_4-N supplementation to any of the treatments was not related to the rates of C losses in *subsoil*: i.e., the mineral fertiliser only soil (S_{POS}) lost half of the C that was lost under S_{RAS}. As mentioned above, the sum of nutrients provided by the fertiliser and through soil mineralization led to the larger yields in all fertilized *subsoil* treatments. The net loss of C may be interpreted as positive priming effect in *subsoil* that led to significant C losses, a hypothesis yet to be tested. Priming has been reported to degrade resistant soil carbon more effectively than active soil carbon (Chen et al., 2022). According to this, in our experiment the losses of OC in *subsoil* and accumulation in the *topsoil*, could suggest that the *subsoil* might harbour more resistant organic carbon, that usually has larger C:N:P ratios and which upon mineralisation leads to respiratory losses of C.
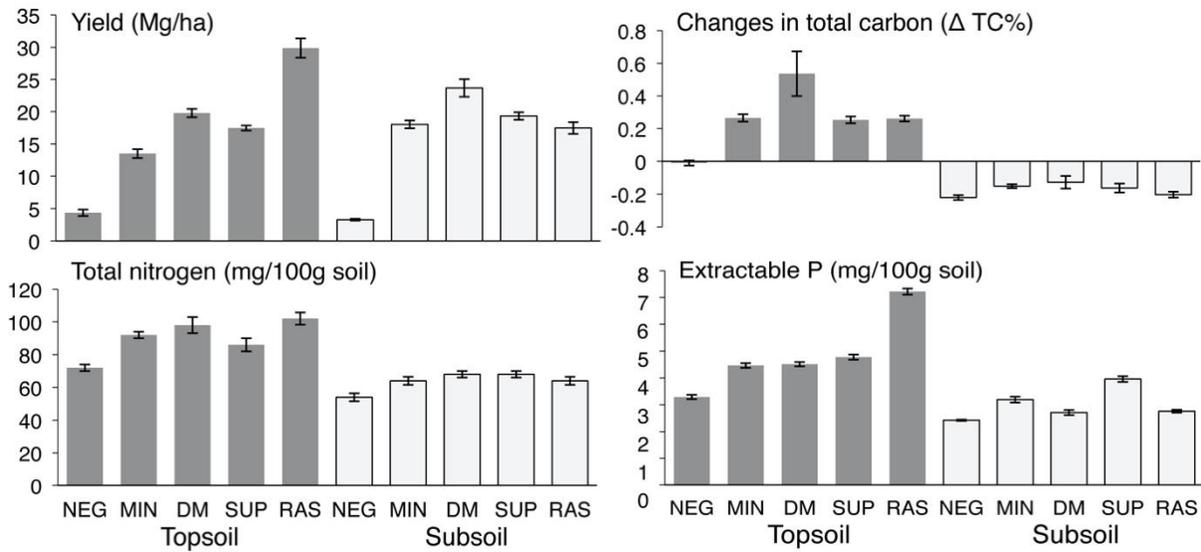
Figure 3. Yield, total soil nitrogen (mg/100g dry soil), Olsen Extractable soil P (mg/100g dry soil), and changes in soil total carbon (Δ TC%) at the conclusion of experiment: DMN, dairy manure, MIN, mineral fertilizer, Neg, negative control, RAS, recirculating aquaculture solids, and SUP, recirculating aquaculture solid tank supernatant.

P dynamics in boreal soils is not always reflected in sustainable nutrient management plans (Kedir et al., 2021). Availability of P is depressed by decreased pH and increased Al and Fe contents (Grand et al., 2014). P acquisition efficiency was greatest for T_{RAS}, the treatment that seems to have led to significant increases in N possibly through stimulating microbially driven processes. At harvest, the extractable P was significantly greater in the *topsoil* (Table 6). Podzolic subsoils favour P retention in unavailable forms (Grand & Lavkulich, 2011). Considering the diminished P uptake by plants from *subsoil* it is possible that phosphatase activity is lower in this soil. This was expected as from the initiation of the experiment the *subsoil* was in a more P deficient state than *topsoil* (Table 1).

An LDA allowed for a visual assessment of the variance structure in the data set (Figure 2). The drastic differences and alternative soil carbon accumulations are reflected in the grouping of S_{RAS} and T_{RAS}; model differences and effects are summarised in Table 6. In turn the plant response appeared to be a result of the soil interacting with the nutrient source that either amplified or dampened productivity.

Table 7. Linear Model outputs of final plant response and soil nutrient dynamics the interactive effects of soil depth and nutrient source in boreal podzol

Predictors	Yield			Change in Soil C			Final Soil P			Final Soil N		
	Estimates	CI95	p	Estimates	CI95	p	Estimates	CI95	p	Estimates	CI95	p
Intercept	5.42	1.65 to 9.19	0.006	0.2	-0.02 to 0.42	0.07	4.15	3.75 to 4.56	<0.001	90	76.59 to 103.41	<0.001
Soil Depth	-1.07	-3.46 to 1.32	0.369	-0.21	-0.35 to -0.07	0.004	-0.86	-1.12 to -0.61	<0.001	-18	-26.48 to -9.52	<0.001
DMN	10.45	5.12 to 15.79	<0.001	1	0.69 to 1.31	<0.001	2.16	1.59 to 2.73	<0.001	38	19.04 to 56.96	<0.001
RAS	36.9	31.56 to 42.23	<0.001	0.52	0.22 to 0.83	0.001	7.54	6.96 to 8.11	<0.001	50	31.04 to 68.96	<0.001
SUP	10.17	4.83 to 15.51	<0.001	0.47	0.16 to 0.78	0.004	1.44	0.87 to 2.01	<0.001	14	-4.96 to 32.96	0.143
Mineral	3.56	-1.78 to 8.90	0.185	0.48	0.17 to 0.79	0.003	1.58	1.01 to 2.16	<0.001	30	11.04 to 48.96	0.003
Soil depth: DMN	4.97	1.60 to 8.35	0.005	-0.45	-0.65 to -0.26	<0.001	-0.94	-1.30 to -0.57	<0.001	-12	-23.99 to -0.01	0.05
Soil depth: RAS	-11.36	-14.73 to -7.98	<0.001	-0.25	-0.45 to -0.06	0.012	-3.6	-3.96 to -3.24	<0.001	-20	-31.99 to -8.01	0.002
Soil depth: SUP	2.94	-0.44 to 6.31	0.086	-0.2	-0.40 to -0.01	0.039	0.05	-0.31 to 0.41	0.791	0	-11.99 to 11.99	1
Soil depth: Mineral	5.59	2.22 to 8.97	0.002	-0.21	-0.40 to -0.01	0.039	-0.41	-0.77 to -0.05	0.029	-10	-21.99 to 1.99	0.1
Observations (n)	50			50			50			50		
R^2; R^2 adjusted	0.95; 0.94			0.87; 0.84			0.98; 0.98			0.87; 0.85		

4. Conclusions

While the two soils, topsoil and subsoil were surprisingly similar in their make-up as measured through standard soil quality parameters, the responses of the plants to the two soils receiving the same nutrient sources were obviously distinct. The *topsoil* affected the immediate availability of nutrients, possibly due to nutrient fixation in soil microbial biomass, and thus led to a slower initial plant growth, but favoured availability over the longer term, allowing continuing plant growth. The *topsoil* also favoured accumulation of soil carbon, thus behaving as a healthier, more sustainably productive soil. Detailed insights into the functional status of soil microbiomes for converted podzolic soils are clearly necessary to understand the levers of long-term fertility build-up and sustainability of nutrient management. From a practical point of view these results also raise the question of management advising. In a LUC event subsurface originating soils will be more responsive to the addition of mineral fertiliser, and thus, arguably more fertile in a conventional management. The utility of organic fertiliser as source of nutrients and agricultural production after conversion of boreal Podzols will be dependent on the post-conversion status of the soil as affected by the conversion approach, i.e., depth of removal of surface soil, extent of soil layer mixing through the initial tillage. While the application of organic wastes as fertilisers to a boreal Podzol is recommendable, this experiment indicates that soil depths represented in the converted land dictate how effectively organically derived nutrient application will impact the agroecosystem.

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Chapter 3: Biomass stoichiometry for tall fescue grown on a boreal Podzol as affected by soil layer and fertiliser type

Byline: Plant economic traits in boreal podzol land conversion could be indicator of soil quality and sustainable development; edaphic alterations may impact the economic traits of plants in boreal podzol

Abstract

Climate change projections are changing perspectives on boreal ecosystems with a focus shifting to the potential necessity for agricultural productivity. One of the dominate soil types in the boreal region are Podzols that are known for their low organic matter (OM) content. OM a common and well defined indicator of soil quality. Stoichiometric ratios have been applied in ecology to measure the dynamics of ecosystem functions and may be employed to enhance the understanding and monitoring of SOM based soil quality assessments. This work set out to identity the potential application of stoichiometric ratios in boreal podzol land use conversion events where organic matter content may not be the ideal indicator of soil quality. Two soil depths (0-15cm [topsoil] and 15-30cm [subsoil]) were collected and amended with locally relevant organic waste fertilisers (dairy manure [DMN], recirculating aquaculture system supernatant [SUP] or slurry [RAS]), with mineral fertilisation as control. Tall fescue served as a model for biomass accumulation of C:N:P and was used to measure the transfer of N and P to plants as a measure of functional capacity of the soil to sustain continued production. Starting available nutrient concentrations were equalized as necessary with mineral N and P fertilisers. Noted differential C:N:P ratios in test crop biomass were due to interactive effects of soil depth and nutrient source; while the test crop was provided with the same apparent quantity of nutrients, the plant biomass C:N:P ratios were found to differ

significantly. P uptake and availability proved to subscribe to the law of the minimum with respect to subsoil interactive effects with predominantly organic sourced P. The use of plant tissue nutrient ratios was able to help identify the limiting components of the system providing evidence for further exploration in to field based settings. Boreal podzol soil layers led to differential nutrient uptake and utilisation of the same fertilisers, an observation of interest for the sustainable nutrient management in podzol when soil layers are variably mixed at land use conversion.

1.0 Introduction

1.1 Status of boreal ecosystems and projected land use requirements

Previously unexperienced and alarming accelerated rates of warming in northern regions are now a reality. An active component of climate change mitigation strategy involves the local production of food to shorten C dependent supply chains (Kok et al., 2022; Roussin et al., 2015). Regions with northern boreal forests previously not known for agricultural activity will require land use conversion (LUC) followed by land use intensification (LUI) to enhance local production (Unc et al., 2021). LUC risks immediately involve the increase in soil abiotic factors that then influence biotic C cycling and subsequently the mineralization of N and P (Cui et al., 2020). Conversion to agriculture can promote food production relevant services but at the cost of other ecosystem functions and services (Allan et al., 2015) shifting the definition of soil quality thus impacting how soil heath and sustainability will be measured (Pretty, 1995).

1.2 Risks associated with land use conversion on Podzols

In a natural state the dynamics of nutrient availability in Podzols are impacted by the soil acidity, soil moisture, permeability, surface run off and erosion modifying carbon and nitrogen kinetics (Altdorff et al., 2017). Plant nutrient availability is dependent on the cycling between available

and unavailable pools (Bracken et al., 2015). When land use is altered associated cycles are impacted. Specific environmental risks associated with the conversion from forest to agriculture on boreal podzols, include the leaching of organic compounds to lower horizons and the mobilization of the Al and Fe in the upper horizon (Hughes et al., 1990).

1.3 Bennifical Management Practices for new agricultural land

Changes in land use will modify the nature of nitrogen cycling in the environment and the way in which N fertilisers are added will both influence the farm productivity and thus agricultural development. Phosphorus (P), a critical element for metabolic regulation of all living cells, is a non-renewable and depleted resource (Kedir et al., 2022) and is commonly the limiting factor for terrestrial plant productivity (Gleeson & Tilman, 1992, Fay et. al, 2015). Plant available macronutrients that carry negative charges are phosphates, nitrates and sulphates all are most strongly influenced by the solubility of anion rich minerals (de Bang et al., 2021). Another factor specific to the region is that anions are retained in Podzols due to pH values, under the point of zero charge (pzc) (Schwertmann & Fechter,1982) values for Fe and Al oxides (Porras et al., 2017). Boreal podzol P dynamics are complex: a particularity is the high P storage capacity due the retention in complexes with Fe and Al, corresponding to high buffering capacity for acidity. If not made unavailable phosphorus addition to farm soils can accelerate litter decomposition stimulating the rate of N mineralization leading to increased availability to plants (Bracket et al 2015). This can cascade into an increased rate of OM mineralization altering anticipated priming effects (Siles et. al., 2022). Given the naturally low organic matter content due to cold conditions that limit the mineralization of organic matter and the high infiltration rates of precipitation the amount of dissolved organic carbon (DOC) and other soluble species can be lost from the soil environment. The low concentrations of organic matter in new farmed podzols, especially under conventional

tillage post LUC, may limit SOM's utility as a bridge between the abiotic and biotic factors impacting the agricultural productivity of LUC scenarios. Elemental ratios, including limiting nutrients, provide insights into the status of soil plant relationships. It may be assumed that each photosynthetic plant, according to its physiological state, has an ideal stoichiometric ratio but that can be altered due to environmental conditions. The Redfield ratio was first assessed for algae in oceans (Redfield, 1958) but was then variably applied to discussions on the physiology of terrestrial plants. The relationship between elements like C, N and P expressed as a ratio can be employed to infer the organism's biochemical composition and its physiological state (Čapek et al., 2018). The C:N:P ratio can also provide insight into the status and stability of the ecosystem. A major application of nutrient ratios is the ability to measure the impact and response of environmental change or human intervention though plant tissue compositions. Ratios can be expressed as mass ratio or an atomic ratio. The use of atomic or molar ratios is most common in plant physiology research due to the relevance to actual stoichiometric relationships (Zechmeister-Boltenstern et al., 2015).

1.4 Stoichiometric stability in an agroecosystem

Given the conservation of nutrient composition in plant tissue with associated function the application of stoichiometry in agronomy may assist in fertiliser management modeling yet caution needs to be taken as there is variability associated with different crops and crop produce (Sadras, 2006). The relationships between plant and soil are not exclusive but dependent on facilitation form microbes in the environment as soil microbes regulate fluxes in soil stoichiometry and allow for abiotic concentrations to estimate biotic interactions (Li, et al., 2019). The inclusion of N:P ratios into community and ecosystem models may enhance their predictive utility for change and mitigation (Güsewell, 2004). The use of stoichiometric ratios provides a valuable link between

cellular and ecosystem process allowing decision makers to use extrapolations of relative ratios at organismal levels to support predictions of global changes on ecosystem functioning (Leroux, et al. 2017). General drivers of instability in ecosystems and agriculture can functionally be attributed to the impacts of human activity. Abiotic factors like precipitation and temperature are affected by global climate change, thus influencing biological components. In nature the excessive loading of nutrients result in the loss of biodiversity cascading into the loss of services being delivered that include clean water, fertile soils and nutrient cycles transferring through trophic levels (Guignard et al., 2017). Being a driver for plant growth and microbial metabolism the availability of nitrogen to microbes will affect the decomposition rate of carbon in the soil thus impacting nitrogen availability to plants. Soil C:N:P ratios can indicate the relative stability of SOM, availability to microorganism and thus availability of mineralizable nutrient to plants, all stimulating processes that allow for the uptake of other essential nutrients (Li, et.al., 2019).

Agroecosystems can be directed away from stability from continuous application of nutrients without knowledge on how these nutrients are being cycled and used. Conditions of over application can impact microbial community diversity and in turn activity. In a Finnish study, results indicate that after forest clear cutting the annual deposition of organic nitrogen decreased with a concomitant increase in available N (NH_4^+, NO_3^-). The ability of an ecosystem to retain nutrients is crucial to sustainability where balances in availability will impact coupled system (Piirainen, 2002).

1.5 Autotrophs in ecosystem stoichiometry

Lands that are destined for conversion to agriculture will require the application of nutrients to achieve the desired production. Measuring the efficiency of such applications can be difficult to carry out in a manner that involves the ecosystem as a whole; the nutrient ratios in plant tissues

can offer insight into the interactive response between soil, microbes and plant to the addition of nutrients like N and P (Güsewell et al., 2003). Management practices such as tillage, crop profile, crop rotation and land use history all affects nutrient utilization in agriculture. Long term monitoring is required to confidently quantify the impact of management practices/system. There is a potential in applying soil and plant nutrient stoichiometry as a tool for early detection of impacts due to management or land use change (Weih et al., 2016). Nutrient ratios can provide insight into the limiting status of a nutrient; it is important to note that the ratios identify limitations not the concentration of single nutrients (Koerselman & Meuleman, 1996).

Autotrophs do not always maintain the stoichiometric balance in tissues as the environment will influence mechanisms for nutrient questions strategies based on the variable resource supplies. (Sterner & Elser, 2002). The law of the minimum states that plant growth will be limited by the least available essential nutrient. The use of ratios in foliar tissue may be used as sensitive proximate indicators of nutrient limitation to plant growth and extend as a tool to measure the impact of nutrient addition on ecosystem health (Tessier, 2003) and thus quantify the services being delivered. N:P ratios cannot always clearly describe plant physiological state as leaf senescence or luxury consumption can muddle the interpretation of nutrient demand and utilisation (Čapek et al., 2018). The stoichiometry of a plant is in part affected by the nutrient availability in the soil and the ability of the crop to acquire nutrients thus leading to the concentration of element in plant tissue. The growth rates increase linearly with plant nutrient concentration up to a point where the acquired nutrients surpass the needs of the plant and capabilities creating a state of luxury (Weih et al., 2016). Sampling plant tissues and soil is a well-established practice in fertility management; the same data can be used in terms of ecological stoichiometry for a better interpretation of sustainable management.

If nutrient ratios in plants are expected to be used as a measure of ecological sustainability and soil quality in boreal region the evaluation of the ideas needs extensive testing to develop a baseline for measurement to serve as a point of reference that can also be linked to plant physiological activity. In an attempt to link theories of soil quality and ecological stoichiometry a greenhouse pot study was conducted. This experiment included sampled topsoil and subsoil from an area slated for LUC from a *Larix laricina* seed orchard to agricultural use. Mineral and organic fertilisers were added to each soil depth, with tall fescue as the indicator plant. Concentrations of C:N:P where measured in plant tissues and soils in a short term pot experiment to assess the utility of nutrient ratios as a predictor for soil quality based on agronomic management decisions. The topsoil (0-15cm) and subsoil (15-30cm) were tested separately to first identify any variability in the plow layer an important first step in building predictions on how LUC event will impact nutrient cycles and fertility management.

Hypothesis: Variable concentrations of nutrients will accumulate in tall fescue, affected by the combination of soil depth and nutrient source; the use of nutrient ratios would provide contextual and transferable evidence of any limitation in the system regardless of the complexities in nutrient cycle dynamics.

2.0 Materials and Methods

See materials and methods in chapter 2

2.1 Plant and Soil Molar Concentrations

Molar concentrations of plant and soil tissue were determined by the quantity of a given element in 100g of tissue of soil divided by the molar mass of given element.

3.0 Results

3.1. Soil pH

At harvest, soil pH ranged from 4.9 to 6.1, a shift from the starting pH of 5.3. An ANOVA indicated significantly different final pH values depending on treatments that differed in nutrient source and soil depth. Interestingly there seemed to be an interaction between the organic nutrient source and the soil depth as DMN and RAS grouped differently in top and subsoil counterparts (Figure 1.). A general linear model exploring the interactive effect of soil depth and nutrient source on pH resulted in a constant coefficient of 5.62 with an R-Sq of 89.9%; the model output identified that mineral nutrient source would have a negative impact on pH i.e., acidification of -0.66 (p >0.001) and DMN and RAS would influence the pH by 0.12 and 0.06 although not significantly. The depth of the soil was found to significantly influence the soil pH with a coefficient of 0.24 (p= 0.006); further evaluation indicated that interactive effect between soil depth and nutrient source was significant in S_{RAS} and S_{SUP} (coef of -0.76, p>0.001 and coef of -0.22, p =0.067). The soil pH glm identified that soil, nutrient source and the interactive effect between soil and nutrient source to all have significant differences (p values 0.006, >0.001, >0.001) identifying chemical differentiation between the treatments tested (Supplementary Table 4).
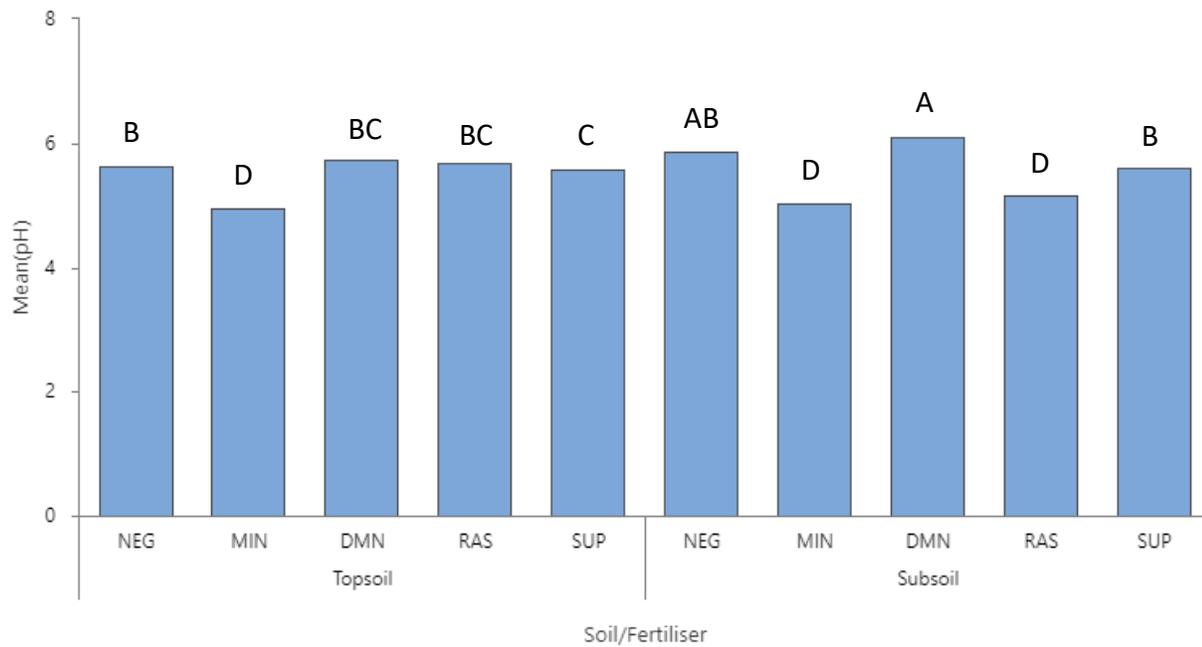
Figure 1. Mean Soil pH at conclusion of experiment, letters above bars are Tukey pairwise comparison. DMN, dairy manure, MIN, mineral fertilizer, NEG, negative control, RAS, recirculating aquaculture solids, and SUP, recirculating aquaculture solid tank supernatant. Top soil (0-15cm) and Subsoil (15-30cm) collected from boreal podzol slated for LUC.

3.2 Principal Component Analysis (PCA): plant growth and nutrient acquisition
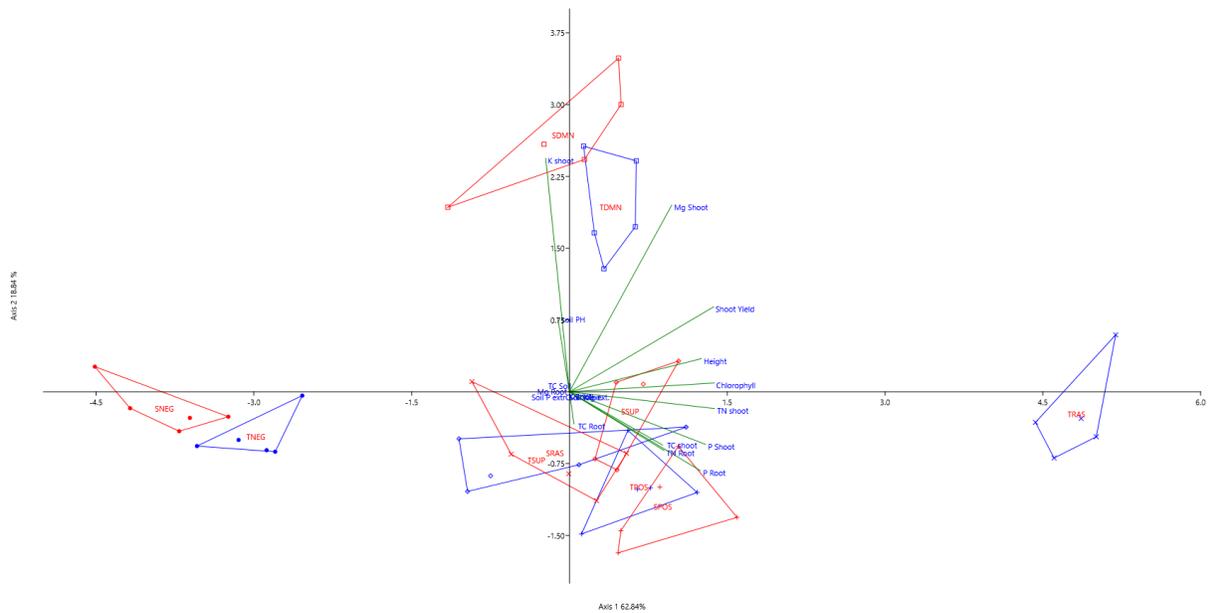
Figure 2. Moles/100g of Soil and Plant Tissue PCA image axis 1 62.84% axis 2 18.84%, blue shapes (topsoil),red shapes (subsoils), circles are negative controls,+ signs are positive control, hollow square are equivalent to DMN, X represents RAS and diamonds shapes the SUP treatments. Eigen vectors included, yield, height, chlorophyll soil TC, soil TN, soil extractable P,K,Mg, root and shoot elemental concentrations of C,N,P,K and Mg.

3.3 Plant Physiology and nutrient concentration correlations

Table 1. Correlation between elemental Nutrient stoichiometry correlation scores as R values are given as decimals, CI 95% intervals

	Shoot Biomass	Root Biomass	Sh. C:N	Sh. C:P	Sh. N:P	R. C:N	R. C:P	R. N:P	Chlorophyll	Soil C:N
Root Biomass	0.501*** (0.259, 0.684)									
Sh. C:N	-0.525 *** (-0.701, -0.289)	-0.147 (-0.409, 0.137)								
Sh. C:P	-0.365** (-0.584, -0.096)	0.008 (-0.271, 0.286)	0.640*** (0.440, 0.779)							
Sh. N:P	0.132 (-0.152, 0.396)	0.128 (-0.156, 0.392)	-0.343* (-0.567, -0.071)	0.493*** (0.249, 0.678)						
R. C:N	-0.220 (-0.470, 0.062)	0.036 (-0.245, 0.311)	0.283* (0.005, 0.520)	-0.092 (-0.361, 0.192)	-0.412** (-0.619, -0.150)					
R. C:P	-0.255 (-0.498, 0.025)	-0.182 (-0.438, 0.102)	0.287* (0.009, 0.523)	0.045 (-0.236, 0.319)	-0.238 (-0.484, 0.043)	0.910** (0.847, 0.948)				
R. N:P	-0.090 (-0.360, 0.193)	-0.437** (-0.638, -0.181)	-0.080 (-0.351, 0.203)	0.374** (0.107, 0.591)	0.573*** (0.350, 0.734)	-0.300* (-0.534, -0.024)	0.050 (-0.231, 0.324)			
Chlorophyll	0.799*** (0.670, 0.882)	0.352* (0.082, 0.574)	-0.741*** (-0.845, -0.582)	-0.519*** (-0.697, -0.281)	0.192 (-0.091, 0.446)	-0.202 (-0.455, 0.081)	-0.222 (-0.472, 0.060)	-0.018 (-0.295, 0.262)		
Soil C:N	-0.107 (-0.374, 0.176)	0.078 (-0.204, 0.349)	0.231 (-0.050, 0.479)	-0.070 (-0.342, 0.212)	-0.333* (-0.559, -0.060)	0.307* (0.031, 0.539)	0.269 (-0.011, 0.509)	-0.240 (-0.486, 0.041)	-0.100 (-0.368, 0.184)	
SOM	0.221 (-0.061, 0.470)	0.267 (-0.012, 0.508)	-0.154 (-0.414, 0.130)	-0.396** (-0.608, -0.132)	-0.316*** (-0.546, -0.041)	0.227 (-0.054, 0.476)	0.105 (-0.179, 0.372)	-0.344 (-0.568, -0.073)	0.276 (-0.003, 0.515)	0.796*** (0.665, 0.880)

*p value < 0.05 > 0.01

**p value < 0.01 > 0.001

***p value < 0.001

Below person correlation scores CI 95% intervals are in brackets, R.(root tissue, below), Sh. (shoot tissue, above soil)

Table 2. C:N:P elemental ratios in plant tissue and C:N in soil, SOM, and plant biomass response at harvest, collected from boreal podzol pre land use conversion

Treatment		Molar Nutrient ratios			Biomass (kg/m^2)				Chlorophyll at Harvest SPDI[#]
		Shoot C:N:P	Root C:N:P	Soil* C:N	SOM (% dry soil)	Shoot	Root	Shoot:Root Ratio	
Depth 0-15cm	T_{NEG}	1282:36:1	764:21:1	15.34 a,b,c	1.63 c	0.153 d	0.227 a	0.692 a	2.53 d
	T_{POS}	970:37:1	739:23:1	15.503 a,b,c	2.09 b	0.33 c	0.358 a	1.018 a	29.33 a,b
	T_{DMN}	1119:42:1	844:24:1	17.73 a	2.55 a	0.489 b	0.875 a	0.862 a	27.86 b
	T_{RAS}	539:35:1	364:17:1	13.96 b,c,d	2.09 b	0.716 a	0.631 a	1.521 a	36.26 a
	T_{SUP}	1181:31:1	875:22:1	16.51 a,b	2.07 b	0.483 b	0.466 a	1.204 a	20.37 c
Depth 15-30cm	S_{NEG}	1469:35:1	1040:24:1	13.57 b,c,d	1.08 d	0.097 d	0.153 a	0.717 a	1.35 d
	S_{POS}	964:39:1	677:24:1	12.66 c,d	1.20 d	0.472 bc	0.489 a	1.199 a	30.42 a,b
	S_{DMN}	1363:47:1	987:28:1	12.33 d	1.24 d	0.568 ab	0.744 a	1.081 a	23.44 b,c
	S_{RAS}	1556:66:1	953:34:1	11.74 d	1.11 d	0.472 bc	0.534 a	1.396 a	26.71 b,c
	S_{SUP}	1065:38:1	812:24:1	11.73 d	1.18 d	0.511 b	0.409 a	1.463 a	25.99 b,c

Shared letters associated with means indicate no significant difference; columns with no Tukey pairwise comparison have results available in Supplementary Table 3

*Soil P in ratio omitted as measured concentrations where Olsen extractable P and not total P

Table 3 Stoichiometric Relationship Linear Regression Analysis C:N:P ratios

	Shoot tissue						Root Tissue						Soil	
	C:N		C:P		N:P		C:N		C:P		N:P		C:N	
	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P
Constant	26.26	>0.001	1281.9	>0.001	35.63	>0.001	37.15	>0.001	739.4	0	20.64	0	0.163	>0.001
T_{MIN}	-10.66	0.002	-312	0.014	1.29	0.712	-5.13	0.014	-24	0.843	2.47	0.513	-1.381	0.851
T_{DMN}	-10.43	0.002	-162	0.188	6.59	0.065	-2.05	0.312	81	0.506	3.42	0.366	1.174	0.118
T_{RAS}	-21.41	>0.001	-743	>0.001	-0.78	0.823	-15.42	>0.001	-399	0.002	-3.77	0.319	1.905	0.182
T_{SUP}	1.47	0.651	-101	0.41	-4.88	0.168	3.18	0.12	111	0.361	0.99	0.793	-1.753	0.033
S_{NEG}	5.9	0.074	187	0.13	-1.04	0.767	5.62	0.008	276	0.028	3.63	0.337	-2.675	0.049
S_{MIN}	-12.02	0.001	-318	0.012	3.01	0.392	-8.9	>0.001	-87	0.476	3.29	0.384	-3.007	0.004
S_{DMN}	-7.58	0.023	81	0.508	30.37	>0.001	-1.35	0.504	224	0.071	7.64	0.048	-3.599	0.001
S_{RAS}	-13.39	>0.001	274	0.03	11.64	0.002	-8.87	0	190	0.124	12.86	0.001	-3.611	>0.001
S_{SUP}	-9.07	0.007	-217	0.081	2.83	0.421	-3.19	0.119	48	0.691	3.37	0.372	0.163	>0.001
R-sq	0.7388		0.7258		0.7417		0.813		0.5335		0.3881		0.7059	

Table 4. Linear Regression analysis with interactive function between soil depth and fertiliser

	Shoot Tissue						Root Tissue						Soil	
	C:N		C:P		N:P		C:N		C:P		N:P		C:N	
	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P	Coef	P
Constant	36.92	>0.001	1281.9	>0.001	35.63	>0.001	37.15	>0.001	763.5	>0.001	20.64	0	15.34	>0.001
Subsoil	5.9	0.074	187	0.13	-1.04	0.767	5.62	0.008	276	0.028	3.63	0.337	-1.753	0.049
Min	-10.66	0.002	-312	0.014	1.29	0.712	-5.13	0.014	-24	0.843	2.47	0.513	0.163	0.851
DMN	-10.43	0.002	-162	0.188	6.59	0.065	-2.05	0.312	81	0.506	3.42	0.366	-1.381	0.118
RAS	-21.41	>0.001	-743	>0.001	-0.78	0.823	-15.42	>0.001	-399	0.002	-3.77	0.319	1.174	0.182
SUP	1.47	0.651	-101	0.41	-4.88	0.168	3.18	0.12	111	0.361	0.99	0.793	1.905	0.033
Interactive Regression analysis between Soil depth and Fertiliser														
Sub: DMN	-3.05	0.507	56	0.746	6.09	0.223	-4.92	0.09	-133	0.439	0.59	0.912	0.13	0.918
Sub: RAS	2.12	0.643	830	>0.001	32.19	>0.001	0.92	0.747	313	0.074	13	0.018	-3.02	0.018
Subs SUP	-16.44	0.001	-304	0.084	8.74	0.083	-12	>0.001	-339	0.054	-1.25	0.815	-3.76	0.004
Sub: MIN	-7.27	0.118	-194	0.265	2.75	0.579	-9.4	0.002	-339	0.054	-2.81	0.598	-1.08	0.38
R-sq	0.739		0.726		0.789		0.813		0.534		0.388		0.706	

4.0 Discussion

4.1 Soil pH and elemental chemistry

Soil pH governs soil chemical and biological process. Significant differences in soil pH were noted at the conclusion of the experiment (figure 1); specific interactive effects were noted between the subsoil and RAS (Supplementary Table 4) that resulted in the significantly different pH reduction compared to the topsoil and RAS. The soil-depth dependent divergence in soil acidification for the different nutrient sources is of interest for recommendations on the use of mineral or organic fertilizer to newly converted lands. The apparent pH buffer capacity found in the subsoil (i.e., as inferred from measurements of changes) appeared to be weak; the increased uptake of mineral N is one likely explanation for the drop in pH. When the treatment mixtures (chapter 1 table 2) are considered, it can be noted that the largest amounts of mineral N were applied to S_{MIN} and S_{RAS} which upon statistical analysis showed a similar pH (i.e., Tukey pairwise comparisons). This change in pH offers a potential explanation involves the nitrification of ammonium and thus a release of H^+ ions into solution (Weber and Gainey, 1962). Change in acidity associated with mineral application of N could have also resulted from deprotonation upon ammonium uptake by plant roots; the mineral nature of the applied nutrient does not require microbial mineralization for plant availability (Bolan et al.; 1991). The observed soil acidification associated with mineral N application was not found to be exclusive to depth or N amounts, but the source. DMN application (where most N was applied in organic forms and P supplemented in mineral form) provided evidence that sourcing N derived from organics may be an effective BMP for converted lands.). The PCA (figure 2) indicated that plant shoot K content was linked the application of dairy manure to both the top and subsoils. Given that ionic K is taken up through the root system through mass flow, there must have been a higher concentration of K in the soil solution of treatments that received DMN. The Ca found in the could have exchanged with adsorbed K on soil colloids, clay

and OM; the shoot tissues for both DMN top- and sub-soils had molar Ca concentrations similar to that of the negative control a result that was exclusive to DMN treatments. The addition of both Ca and K from DMN may in boreal LUC may help balance macronutrient plant acquisition. The role of calcium in the plant walls provide structure and resistance to lodging in support of the cell wall and vacuole (water retention). For commercial grain growth on new lands this may be a point of interest for future research as DMN is commonly applied to boreal podzols in the region. The initial K concentration in DMN was higher from the start (methods table 1) which aligns logically with the information in the PCA image (Figure 2) and the significantly higher mean concentration in plant shoot tissue (Supplementary Table 2). Given that the Mg shoot concentration of the T_{DMN} and S_{DMN} where not negatively impacted (Supplementary Table 1) the excessive availability of K due to DMN application may not be of concern. Excessive K uptake might interfere with uptake of Mg; however, no Mg deficiencies were observed (Supplementary Table 2). The plant acquisition of individual nutrients is of value when assessing soil nutrient availability, yet information is limited to generalized biochemical status of the plant like photosynthetic activity. Calculated nutrients ratios allow using elemental measurements to inform on more complex in-planta interactions.

4.2 Soil C:N and SOM:

The soil C:N ratio (C:N) was related, expectedly, to the SOM concentrations. The stability of nutrient retention in soils will be dependent on the associated species and status of carbon cycle compounds in the soil for example at high C:N the N is locked up in microbial biomass; increasing the N proportion makes carbon more vulnerable to respiratory releases (Audette et al., 2021).

The C:N ratio of many soil amendments provides an indication on the stability of organic matter and potential availability if organic matter nutrients to soil microbiota. Soil C:N ratio was highest in the DMN topsoil treatment and generally lowest in the subsoil; linear regression modeling confirmed that interactive effects between RAS and SUP when mixed with the subsoil resulted in a lower C:N (table 3, table 4). Although mean comparisons did not distinguish a treatment with an exclusive relationship with the SOM, the subsoil SOM% was consistently lower than for the topsoil counterparts of every fertiliser treatment. This may not be due to excessive N (i.e., low C:N ratio) and might be a signal of respiratory C loss. While the low C:N ratio of the subsoil might suggest N availability plant uptake assessments did not confirm this. Moreover, the reduced photosynthetic activity, as measured by SPAD readings, and a reduction in average biomass compared the higher C:N rations observed in the topsoil treatment, confirmed this. Negative coefficients for the linear regressions between -3.61 and -1.75 (see table 2.0), for subsoil treatments support the observation that a lower C:N ratio in subsoils will be expected. Decline of carbon in the subsoils over such a short period of time raises the question of the impact of soil organic carbon amounts and types on mineralization rates after conversion. The possible priming effect identified in chapter 2 is also reflected in soil C:N dynamics, where a decrease in the ratio in soil might be attributed to a loss of soil C. The dynamic nature of how nutrients moved in this experiment demonstrates the need for further detailed work on the cycling of nutrients in boreal Podzol converted soils. In these cases the stoichiometric relationship between C:N may be a misleading measure of sustainability in boreal podzol LUC; these results indicate that the coupling multiple measurements may prove to be the best way of determining anthropogenic impacts on boreal ecosystems destined for agriculture.

4.2 Conventional measure of soil function reimaged to include plant stoichiometry:

The lowest C:N:P ratio was associated with the highest above ground biomass (Table 2); ANOVA results (Supplementary Table 3) support the strong relationship between lower ratios and an improved plant response. Plant shoot ratios in the topsoil treatments showed lower C:N (r =-0.741, p<.001) and C:P (r =-0.519, P<0.001) ratios that also correlated strongly with high SPAD (table1). SPDI readings can be interpreted as the successful acquisition of necessary nutrients from soil.

Soil quality is the ability of a soil to perform a desired function. When land uses are changed, we compel a soil to alter its functions: as a soil changes its functions from effectively supporting a boreal forest to a functional state effectively supporting an agricultural system one must consider that transition period. The lowest C:N:P was associated with the T_{RAS} treatment's shoot and root biomass, and soil at the experiment's conclusion something that the SOM proportions did not distinguish. While one of the most applied quality indicators in soils right now is the organic matter content it may be argued that the use of stoichiometric ratios may be a more advantageous indicator that captures the physiological response of plants to the environment. The T_{RAS} treatment led to most biomass i.e., the most forage was produced in this treatment. The highest chlorophyll readings and in turn the lowest C:N:P, the strength in the relationships observed between, biomass accumulation, physiological plant activity (SPAD) and C:N:P in this study provide evidence that a boreal podzols productivity could be assessed by the elemental ratios C:N:P in crop biomass. Shoot tissues biomass production simply measured the quantity of carbon assimilated yet the use of nutrient ratios provided a broader indication of the relationship of the plant to its environment. Elemental ratios in living tissue can thus be excellent indicators of physiological states at organismal and cellular levels at the time of sample collection. For example the N:Mg ratio in the shoot tissue aligned with previously identified differences in subsoil and topsoil conditions and correlated moderately well and significantly with N:P (r=0.409, p=0.003) (Supplementary Table

3

6). Visual characteristics can be related to what happens at an elemental level, and thus visual observation is a valuable part of assessing experimental outcomes (Li et al., 2019). Each treatment was under the same environmental conditions leaving soil as a source of variability that was best described by the plant biomass elemental economics (Tillman, 1980) or by rates of use of available elements in the environment. The use of nutrient ratios in plant tissues may be an effective measure to assess crop sustainability in climate change adaptation. If the drivers of plant traits are environmental, it is important to gather the appropriate knowledge on how the fertility of newly converted land will behave in current situations before drastic changes in temperature and precipitation alter knowns. Adaption to climate change cannot be met unless all drivers related to anthropogenic changes are fully understood. A useful tool in measuring mitigation and adaption could be in the response of plants in the ecosystem in question (Koerselman et al., 1996). In this experiment the form of nutrients, as dependent on the type of the source of applied nutrients, drove plant responses. Organically derived N and P will lead to distinct plant performance and also affect other major nutrient and micronutrient profiles. In the boreal region the impact of land use conversion on soil nutrient cycling and conservation is an area that could thus be best quantified with the use of plant tissue ratios and a measure of soil quality and future sustainability.

Limiting Factors and the law of the minimum:

The sustainable intensification of lands can be limited by the scarcest nutrient, in accordance to the law of the minimum. The Redfield ratios presented in Table 2 can describe the best performing treatment: T_{RAS} had the lowest C:N:P ratio indicating that the plant had the best proportional acquisition of P; coupled with largest biomass this led to the best P acquisition by mass, as well. Significantly different C:P ratios (Supplementary Table 1) with exclusive Tukey groupings were

reported for T_{RAS} with the lowest ratio and S_{RAS} with the highest ratio. The C:N ratio of T_{RAS} was also lower yet did not significantly different from SRAS. The N:P ratio again highlighted the scarcity of available P to the plant in the SRAS treatment a condition exclusive to the way nutrients where delivered. P is known to decrease in with depth in podzols as Al and Fe concentrations increase, the chelation of P into metallic crystals make biological use difficult and can result in lost P applied. Regulation mechanisms in plants essentially increase uptake of a deficient nutrient and down regulate the uptake of non-limiting nutrient to maintain appropriate homeostatic relationship between N and P for proper plant function. An example is the storage of P in root tissue as polyphosphate resulting in high P concentrations in situations of high P supply a condition demonstrated in the root tissues of the TRAS treatments (Sadras, 2006). It's possible that the P found associated with the root tissue may not have been available to plant as soil colloids may exist in macropore of root tissue, in a sense the roots where able to reach the soil P but the chemical species of P did not allow for its uptake. As to why the subsoil limited the mineralization of organic P, we cannot speculate; further research is required. By applying simple encompassing theories of elementals acquisition as a measure of function the quality of a boreal podzols productivity can be measured to what in this system may be the most limiting nutrient. With the conversion of lands to agriculture the conservation of upper soil horizons appears to be important in the utilization of organic waste streams, a key pillar of sustainable nutrient management.

5.0 Conclusions:

In the same greenhouse experiment soil and plant tissues were measured for C, N, P concentrations while both shoot and root tissue of plants was exposed to complete mineral panel analysis. Results indicated that the use of nutrient ratios can be an effective measure of soil quality by standardizing insight into plant physical activity and success. The accounting of nutrients through ratios provided evidence for organic mineralization of C, N, P leading to why plant response would have varied between treatments. The experiment provides support for the application of nutrient ratios as quality indicators but also acknowledges the extent of said claims given the complexity of nutrient pools in soils-in-flux amended with nutrient sources with variable kinetics. In the like manner, the success of an experiment can never be regarded as proof of the truth" words of Justus Von Liebig.

Universal indictors of soil quality only exist in broad theory, to measure and understand the quality of a soil the function must be defined and measured with appropriate response. Specificity to site will be the key into relevant measure of soil function. By pairing C:N:P ratios from both soil and plant one can develop a series of data that in time will provide reference to the status of that sight. If boreal lands are going to expected to produce agriculturally it is important that productive be measured with and index that can holistically capture complex cycles, by imploring nutrient ratios those who are going to be providing recommendations on productivity and sustainability will need to have an available tool to communicate the impact of management decisions.

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Chapter 4 : General Conclusions and Recommendations

4.1 General Conclusion

- Climate change and population growth will impact global food systems as we know today; resulting in a northern land shift that will require enhancement to meet demands.

- Conventional best management practices may need alterations due to unexplored LUC/LUI saturations.

- Growth rate reflected and performance of test plant was distinctive of soil depth. Organic and inorganic nutrient source performed differently in top and subsoils, which all can be related to the soil carbon loads at the conclusion of the experiment.

- Soil Carbon in the topsoil had increased while in the subsoil decreased, associated with alternative priming effects in the plow layer of a boreal podzol destined for LUC.

- P question was greatened in the T_{RAS} treatment and poorest in the $S_{RAS.}$ An excellent example of the chemical differentiation between the top and subsoils as also reflected in extractable P concentrations and pH at experimental conclusion.

- The use of elemental ratios (C:N:P) in soil and plant was able to confirm observed trends from physical measurements like growth rate and SPDI readings.

- C:N:P linear regression analysis of plant tissue and soil yielded confident models with significant results, indicating potential for the theories of ecological stoichiometry to be effect soil quality indicators.

- Further calibration and multiple sites would be needed to propose C:N:P ratios as soil quality indicators in boreal podzol LUC and LUI events

4.2 Concluding observations and recommendations

It is recommended that continued work to evaluate boreal Podzol soil depths for potential variabilities in nutrient kinetics in areas of LUC. By measuring the status of soil horizons before and after horizon mixing one may create models that could be of benefit in developing recommendations and best management practices to enhance the sustainable agriculture production. The extension of ideas from non-representative conditions to boreal systems have created a set of expectations that are theoretically inapplicable or in the least not transferable The results of this work suggest that converted lands will respond differently to the additions of organic nutrients and mineral fertilizers dependent on the horizon composition present. The separating of the two soil layers into top and subsoils in the plow layer identify that when mixed the composition of the soil and thus its response to organic amendments will be conducive to the representation of top and sub soils. Further research in this area would benefit form including treatments that represent mixed layers to provide a representative of what future nutrient cycles may reflect post conversion. Another benefit would be to sample the area again and conduct the same experiment to see if uniformity between the subsoil and topsoil has been achieved or if further divergences exist. The continuation of work would not only be of benefit to build on technical recommendations but also enhance the accuracy of using nutrient ratios as quality indicators. To best build an indicator continued testing for robust reference is necessary to make build confidence in both precision and accuracy. The works demonstrated that in controlled conditions the use of nutrient ratios in plant and soil provided insight into why observations in plants may have occurred, a concept commonly employed in basic fertility management. The expansion of tissue analysis and soil testing to encompass agroecosystems is an idea that would be an asset to further develop in boreal podzol agriculture. In general field testing and multiple sites are required to build on the

capacity of the theories provided. In all further research focusing on the sustainable intensification and LUC of boreal podzol deserves a greater attention as the day where dependence on these regions for significant agricultural production may be sooner than later.

Supplementary Data

S Table 1 Molar Ratios of C:N:P in above and below ground plant tissue and soil at harvest of tall fescue crop on two boreal podzol depths

	Shoot			**Root**		
	C:P	**C:N**	**N:P**	**C:P**	**C:N**	**N:P**
T_{NEG}	1281 a,b,c	36.92 a,b,c	35.63 c	763.5 a,b	37.15 a,b,c	20.64 b
T_{MIN}	970 c	26.26 c,d,e	36.92 b,c	739.4 a,b	32.02 c,d	23.11 a,b
T_{DMN}	1119.4 b,c	26.48 cd	42.22 b,c	844 a	35.10 b,c	24.05 a,b
T_{RAS}	538.5 d	15.50 e	34.85 c	364.4 b	21.74 e	16.87 b
T_{SUP}	1181 a,b,c	38.38 a,b	30.75 c	874.9 a	40.33 a,b	21.63 a,b
S_{NEG}	1469.3 a,b	42.82 a	34.59 c	1039.5 a	42.77 a	24.27 a,b
S_{MIN}	963.6 c	24.89 d,e	38.64 b,c	676.7 a,b	28.25 d,e	23.92 a,b
S_{DMN}	1362.9 a,b,c	29.34 b,c,d	47.27 b	987.1 a	35.80 b,c	28.27 a,b
S_{RAS}	1556 a	23.53 d,e	66 a	953 a	28.28 d,e	33.5 a
S_{SUP}	1064.5 b,c	27.84 b,c,d	38.46 b,c	811.8 a	33.96 b,c,d	24.01 a,b

Means that do not share a letter are considered significantly different via Tukey Pairwise Comparison

S. Table 2. Moles/100g macro nutrients found in plant tissue reported as mean mg found in harvested tissue total uptake in plant tissue

Moles/100g found in shoot tissue

	C	N	P	K	Mg	Ca
T_{NEG}	3.45 b,c	$9.82E^{-02}$ c,d	$2.72E^{-03}$ c,d,e	$5.03E^{-02}$ b	$5.81E^{-03}$ c	$6.74E^{-03}$ e,f
T_{MIN}	3.65 a	$1.42E^{-01}$ b	$3.95E^{-03}$ b	$4.39E^{-02}$ b	$6.92E^{-03}$ b,c	$1.12E^{-02}$ c,d
T_{DMN}	3.55 a,b	$1.35E^{-01}$ b,c	$3.22E^{-03}$ b,c,d,e	$7.07E^{-02}$ a	$1.09E^{-02}$ a	$5.65E^{-03}$ f
T_{RAS}	3.60 a,b	$2.35E^{-01}$ a	$6.75E^{-03}$ a	$4.27E^{-02}$ b	$1.27E^{-02}$ a	$2.39E^{-02}$ a
T_{SUP}	3.62 a,b	$9.77E^{-02}$ c,d	$3.19E^{-03}$ b,c,d,e	$3.83E^{-02}$ b	$7.05E^{-03}$ b,c	$8.92E^{-03}$ d,e
S_{NEG}	3.36 c	$7.93E^{-02}$ d	$2.30E^{-03}$ e	$4.97E^{-02}$ b	$6.01E^{-03}$ c	$6.41E^{-03}$ e,f
S_{MIN}	3.66 A	$1.47E^{-01}$ b	$3.84E^{-03}$ b,c	$3.90E^{-02}$ b	$6.01E^{-03}$ c	$1.17E^{-02}$ b,c
S_{DMN}	3.55 a,b	$1.25E^{-01}$ b,c	$2.64E^{-03}$ c,d,e	$7.16E^{-02}$ a	$1.19E^{-02}$ a	$5.80E^{-03}$ f
S_{RAS}	3.63 a,b	$1.55E^{-01}$ b	$2.40E^{-03}$ d,e	$4.40E^{-02}$ b	$5.74E^{-03}$ c	$1.38E^{-02}$ b,c
S_{SUP}	3.63 a,b	$1.34E^{-01}$ b,c	$3.52E^{-03}$ b,c,d	$4.42E^{-02}$ b	$8.36E^{-03}$ b	$1.40E^{-02}$ b

Moles/100g found Root in tissue

	C	N	P	K	Mg	Ca
T_{NEG}	2.562	$6.93E^{-02}$ b	$3.34E^{-03}$ b,c,d	$2.36E^{-02}$ a,b	$9.42E^{-03}$	$7.15E^{-03}$ b
T_{MIN}	3.028	$9.42E^{-02}$ a,b	$4.18E^{-03}$ b	$2.12E^{-02}$ a,b,c,d	$8.78E^{-03}$	$6.63E^{-03}$ b
T_{DMN}	2.858	$8.13E^{-02}$ a,b	$3.36E^{-03}$ b,c,d	$2.61E^{-02}$ a	$1.32E^{-02}$	$7.20E^{-03}$ b
T_{RAS}	2.661	$1.23E^{-01}$ a	$7.39E^{-03}$ a	$1.26E^{-02}$ d	$1.49E^{-02}$	$1.39E^{-02}$ a
T_{SUP}	3.193	$8.02E^{-02}$ a,b	$3.70E^{-03}$ b,c	$2.25E^{-02}$ a,b,c	$8.71E^{-03}$	$6.12E^{-03}$ b
S_{NEG}	2.742	$6.41E^{-02}$ b	$2.64E^{-03}$ d	$2.14E^{-02}$ a,b,c,d	$1.28E^{-02}$	$7.06E^{-03}$ b
S_{MIN}	2.727	$9.64E^{-02}$ a,b	$4.00E^{-03}$ b	$1.59E^{-02}$ b,c,d	$1.14E^{-02}$	$6.60E^{-03}$ b
S_{DMN}	2.499	$7.19E^{-02}$ b	$2.48E^{-03}$ d	$2.35E^{-02}$ a,b,c	$1.72E^{-02}$	$7.24E^{-03}$ b
S_{RAS}	2.724	$9.54E^{-02}$ a,b	$2.76E^{-03}$ c,d	$1.37E^{-02}$ c,d	$1.21E^{-02}$	$7.30E^{-03}$ b
S_{SUP}	3.024	$8.95E^{-02}$ a,b	$3.71E^{-03}$ b,c	$1.97E^{-02}$ a,b,c,d	$8.92E^{-03}$	$6.77E^{-03}$ b

Shared letters associated with means indicate no significant difference, columns with not letters indicate no significant difference between any means

S.Table 3. Micronutrients treatment mean molar concentrators per 100g of biomass in Shoot and Root Tissue

Shoot Micronutrients Moles/100g of tissue

treatment	Cu	Zn	Mn	Na	B	Fe	Al
T_{NEG}	$4.00E^{-06}$ c	$2.00E^{-05}$ b,c	$2.26E^{-04}$ e	$1.76E\text{-}04$ c	$4.00E^{-06}$ a	$5.36E^{-04}$ a,b	$1.43E^{-03}$ a,b
T_{MIN}	$4.00E^{-06}$ c	$4.00E^{-05}$ b	$4.30E^{-04}$ a	$4.60E^{-04}$ c	$3.00E^{-06}$ a,b,c	$2.71E^{-04}$ b	$6.27E^{-04}$ b,c
T_{DMN}	$7.00E^{-06}$ a,b	$3.40E^{-05}$ b,c	$4.00E^{-04}$ a,b	$3.11E\text{-}03$ a,b	$2.00E^{-06}$ c	$2.98E^{-04}$ b	$5.60E^{-04}$ b,c
T_{RAS}	$6.00E^{-06}$ b	$7.30E^{-05}$ a	$2.51E^{-04}$ d,e	$2.79E\text{-}03$ b	$3.00E^{-06}$ a,b	$2.47E^{-04}$ b	$4.48E^{-04}$ b,c
T_{SUP}	$4.00E^{-06}$ c	$3.60E^{-05}$ b,c	$2.33E^{-04}$ d,e	$4.81E^{-04}$ c	$2.00E^{-06}$ b,c	$2.09E^{-04}$ b	$4.26E^{-04}$ b,c
S_{NEG}	$3.00E^{-06}$ c	$1.70E^{-05}$ c	$2.22E^{-04}$ e	$2.14E^{-04}$ c	$3.00E^{-06}$ a,b	$8.34E^{-04}$ a	$2.03E^{-03}$ a
S_{MIN}	$4.00E^{-06}$ c	$3.50E^{-05}$ bc	$3.39E^{-04}$ b,c	$5.42E^{-04}$ c	$3.00E^{-06}$ a,b,c	$3.69E^{-04}$ b	$8.89E^{-04}$ b,c
S_{DMN}	$8.00E^{-06}$ a	$3.60E^{-05}$ bc	$3.68E^{-04}$ a,b,c	$4.25E\text{-}03$ c	$2.00E^{-06}$ c	$1.98E^{-04}$ b	$3.44E^{-04}$ c
S_{RAS}	$4.00E^{-06}$ c	$3.40E^{-05}$ bc	$3.09E^{-04}$ c,d	$3.14E^{-04}$ a	$3.00E^{-06}$ a,b,c	$2.65E^{-04}$ b	$5.44E^{-04}$ b,c
S_{SUP}	$5.00E^{-06}$ c	$3.70E^{-05}$ bc	$2.55E^{-04}$ d,e	$9.22E^{-04}$ c	$3.00E^{-06}$ a,b	$2.87E^{-04}$ b	$5.93E^{-04}$ b,c

Root micronutrients moles/ 100g in tissue

treatment	Cu	Zn	Mn	Na	B	Fe	Al
T_{NEG}	$1.00E^{-05}$ c	$7.50E^{-05}$ b	$4.26E^{-04}$	$1.94E^{-03}$ c	$8.00E^{-06}$	$1.44E^{-02}$	$3.65E^{-02}$
T_{MIN}	$7.00E^{-06}$ c	$8.00E^{-05}$ b	$4.91E^{-04}$	$3.97E^{-03}$ a,b,c	$4.00E^{-06}$	$1.08E^{-02}$	$2.51E^{-02}$
T_{DMN}	$2.40E^{-05}$ b	$7.20E^{-05}$ b	$6.33E^{-04}$	$3.39E^{-03}$ a,b,c	$6.00E^{-06}$	$1.76E^{-02}$	$3.39E^{-02}$
T_{RAS}	$9.00E^{-06}$ c	$1.37E^{-04}$ a	$5.24E^{-04}$	$3.97E^{-03}$ a,b,c	$6.00E^{-06}$	$1.78E^{-02}$	$3.63E^{-02}$
T_{SUP}	$8.00E^{-06}$ c	$8.00E^{-05}$ b	$3.71E^{-04}$	$5.24E^{-03}$ a,b	$3.00E^{-06}$	$9.49E^{-03}$	$2.03E^{-02}$
S_{NEG}	$1.20E^{-05}$ c	$6.10E^{-05}$ b	$5.21E^{-04}$	$2.21E^{-03}$ c	$1.00E^{-05}$	$1.78E^{-02}$	$3.65E^{-02}$
S_{MIN}	$1.00E^{-05}$ c	$7.30E^{-05}$ b	$5.35E^{-04}$	$3.49E^{-03}$ a,b,c	$5.00E^{-06}$	$1.33E^{-02}$	$2.56E^{-02}$
S_{DMN}	$3.20E^{-05}$ a	$6.40E^{-05}$ b	$6.44E^{-04}$	$3.19E^{-03}$ b,c	$7.00E^{-06}$	$2.13E^{-02}$	$3.15E^{-02}$
S_{RAS}	$1.00E^{-05}$ c	$5.90E^{-05}$ b	$5.02E^{-04}$	$2.41E^{-03}$ c	$6.00E^{-06}$	$1.52E^{-02}$	$2.83E^{-02}$
S_{SUP}	$1.10E^{-05}$ c	$7.40E^{-05}$ b	$3.62E^{-04}$	$5.45E^{-03}$ a	$5.00E^{-06}$	$1.16E^{-02}$	$2.45E^{-02}$

Shared letters associated with means indicate no significant difference, columns with not letters indicate no significant difference between any means

S Table 4. General Linear model with Anova Comparing the interactive effect of soil depth and nutrient source on pH

Term	Coef	SE Coef	T-Value	P-Value	
Constant	5.62	0.0585	96.1	>0.001	
Soil					
Subsoil	0.24	0.0827	2.9	0.006	
Nutrient Source					
DM	0.12	0.0827	1.45	0.155	
RAS	0.06	0.0827	0.73	0.472	
SUP	-0.04	0.0827	-0.48	0.631	
Mineral	-0.66	0.0827	-7.98	>0.001	
Soil*Nutrient Source					
Subsoil: DM	0.12	0.117	1.03	0.311	
Subsoil: RAS	-0.76	0.117	-6.5	>0.001	
Subsoil: SUP	-0.22	0.117	-1.88	0.067	
Subsoil: Syn	-0.16	0.117	-1.37	0.179	
Model Summary	S	R-sq	R-sq(adj)	R-sq(pred)	
	0.130767	89.90%	87.63%	84.22%	
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Soil	1	0.144	0.144	8.42	0.006
Nutrient Source	4	2.0056	0.5014	29.32	>0.001
Soil*Nutrient Source	4	1.1448	0.2862	16.74	>0.001
Error	40	0.684	0.0171		
Total	49	6.7722			

S.Table 5. Tukey pair wise comparison for soil pH Grouping Information Using the Tukey Method and 95% Confidence

Soil	N	Mean	Grouping
Sub	25	5.552	A
Top	25	5.516	A

Fertiliser	N	Mean	Grouping
DMN	10	5.92	A
NEG	10	5.74	B
SUP	10	5.59	B
RAS	10	5.42	C
MIN	10	5	D

Grouping Information Using the Tukey Method and 95% Confidence

Soil*Nutrient Source	N	Mean	Grouping
S_{DMN}	5	6.1	A
S_{NEG}	5	5.86	A B
T_{DMN}	5	5.74	B C
T_{RAS}	5	5.68	B C
T_{NEG}	5	5.62	B C
S_{SUP}	5	5.6	B C
T_{SUP}	5	5.58	C
S_{RAS}	5	5.16	D
S_{MIN}	5	5.04	D
T_{MIN}	5	4.96	D

Means that do not share a letter are significantly different.
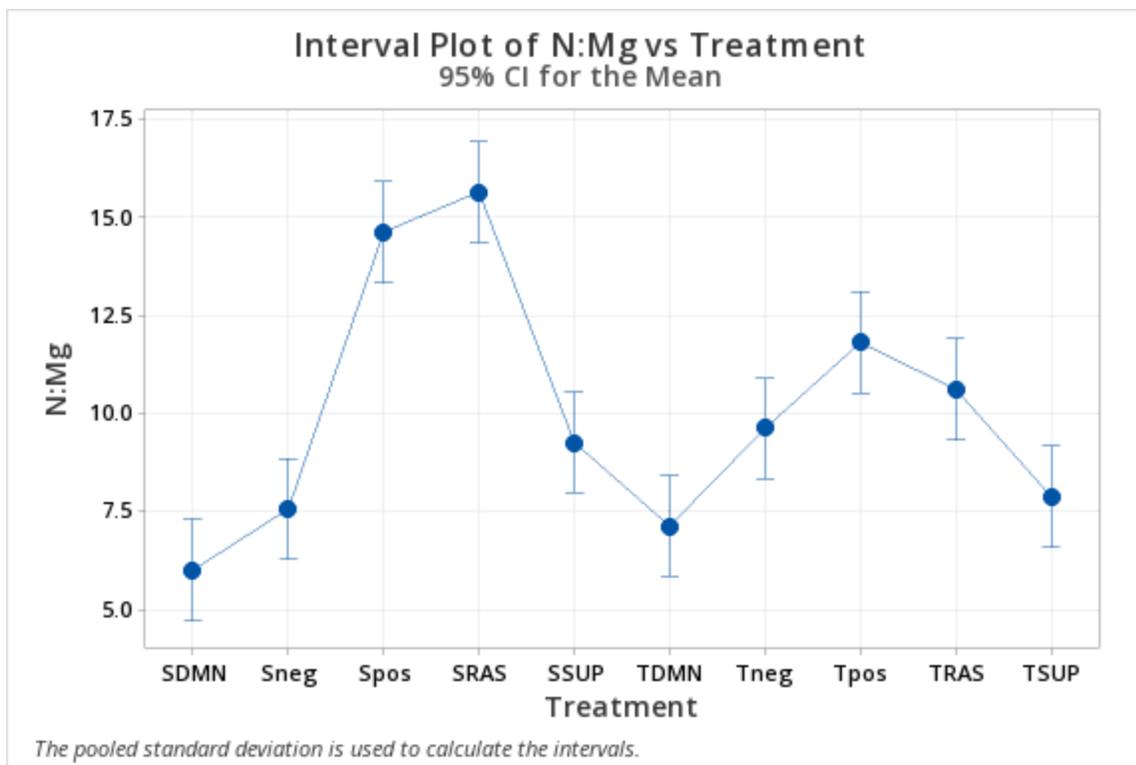
S. Figure 1. Interval Plot of N:Mg ratios as they relate to treatment

Supplementary Table 6. Tukey Pairwise comparison of N:Mg means with SPAD correlation

Grouping Information Using the Tukey Method and 95% Confidence					
Treatment	N	N:Mg Mean	Grouping		
Tneg	5	9.646	cde		
Tpos	5	11.832	bc		
TDMN	5	7.16	ef		
TRAS	5	10.647	cd		
TSUP	5	7.916	def		
Sneg	5	7.6	ef		
Spos	5	14.65	ab		
SDMN	5	6.042	f		
SRAS	5	15.667	a		
SSUP	5	9.291	cde		
Means that do not share a letter are significantly different.					
Correlations N:Mg Chlorophyll 0.318					

S. Table 7. Pairwise Pearson Correlations Chlorophyll, Mg and N

Sample 1	Sample 2	N	Correlation	95% CI for ρ	P-Value
Chlorophyllat Harvest	N:Mg	50	0.318	(0.044, 0.548)	0.024
N:P	N:Mg	50	0.409	(0.148, 0.617)	0.003
N:P	Chlorophyllat Harvest	50	0.192	(-0.091, 0.446)	0.182