

**SOIL PRODUCTIVITY AND SOIL HEALTH STATUS FOLLOWING RECLAMATION
OF PIPELINE CORRIDORS ON AGRICULTURAL LAND**

by

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ABSTRACT

Clemence Muitire, PhD., The University of Manitoba, October 2024. Soil Productivity and Soil Health Status Following Reclamation of Pipeline Corridors on Agricultural Land. Advisors: Dr. Francis Zvomuya and Dr. Inoka Amarakoon

Underground pipeline construction on cropland can reduce crop productivity on the pipeline rights-of-way (ROWs). There is a dearth of information on the recovery of soil productivity with increasing time since reclamation (TSR) of ROWs on cropland. Therefore, the objective of this study was to determine the effect of TSR on the recovery of soil properties and crop yield on underground natural gas pipeline ROWs. We used field and laboratory studies to examine changes in soil properties and soil productivity with increasing TSR. A growth room bioassay using soils collected from ROWs with TSRs of 4 and 10 yr examined the response of ROW soils to fertilizer application in the absence of an operating underground pipeline. Results showed detrimental impacts of the pipelines on soil health and soil productivity, with grain yields 42% lower on the ROWs than on the off-ROWs, even 12 yr after ROW reclamation. Permanganate-oxidizable carbon and autoclaved-citrate extractable protein levels on ROWs approached those on the off-ROW by year 12 following ROW reclamation, while soil respiration (24-h CO₂ burst) recovered within 6 yr of reclamation. Soil penetration resistance in the 20- to 40-cm depth interval was 58-64% greater on the 4.5-yr ROW than on the off-ROW. Saturated hydraulic conductivity was three to four times lower on the 4.5- and 6.5-yr ROWs than on the off-ROW. Effective water conductive macroporosity was 73% lower on the 4.5-yr ROW than on the off-ROW. Six years post-reclamation, the diversity of bacterial and fungal communities on two of the three sites was still lower than on the off-ROW. Nonetheless, all soil and crop attributes showed a steady but slow improvement with increasing TSR. Contrary to the limited crop response to

incremental fertilizer rates in the field study, the growth room bioassay showed a significant biomass yield response to increasing fertilizer rates. These results suggest that persisting crop yield losses observed in the field could be due to the presence of active pipelines. Findings from this research will assist in the formulation of guidelines for pipeline ROW reclamation and the development of land management strategies for the successful restoration of ROW soil productivity.

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FOREWORD

This thesis was prepared in manuscript format according to the Department of Soil Science, University of Manitoba guidelines. The thesis consists of six chapters. Chapter 1 is the general introduction to the study. Chapters 2 through 5 are research chapters prepared as manuscripts for publication in peer-reviewed journals. Chapter 2 focused on the effect of time since reclamation on the recovery of soil health and crop productivity on reclaimed ROWs on cropland. Chapter 3 examined temporal changes in soil physical properties with increasing time since reclamation. Chapter 4 addressed the dynamics of soil bacterial and fungal communities on reclaimed ROWs as affected by the age of the ROWs. Chapter 5 focused on a growth room bioassay that determines if fertilizer management could improve crop yields on ROWs in the absence of active underground natural gas pipelines. Chapter 6 is the overall synthesis which provides a summary of findings presented in Chapters 2 through 5, implications and limitations of the study, and recommendations for future studies.

Publication based on Chapter 2:

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CONTRIBUTION OF AUTHORS

The contributions of all authors to the research chapters presented in Chapters 2 through 5 are as follows.

Chapters 2, 3, and 5 were co-authored by the candidate (first author; conducted experiments; conducted statistical analysis; wrote the original manuscript draft; revised and edited manuscripts), Dr. Francis Zvomuya (provided primary supervision; conceptualized the experiments; acquired project funding; reviewed and edited manuscripts), Dr. Inoka Amarakoon (co-supervised the candidate; conceptualized the experiments; reviewed and edited manuscripts), Dr. Theresa Adesanya (collected data; reviewed and edited manuscripts), and Dr. Afua Mante (reviewed and edited manuscripts). Chapter 2 was published in *Land Degradation and Development*, Chapter 3 was published in *Catena*, and Chapter 5 was published in *Soil Use and Management*.

Chapter 4 was co-authored by the candidate (first author; conducted experiments; conducted statistical analysis; wrote the original manuscript draft; revised and edited manuscripts), Dr. Francis Zvomuya (provided primary supervision; conceptualized the experiments; acquired project funding; reviewed and edited manuscripts), Dr. Inoka Amarakoon (co-supervised the candidate; conceptualized the experiments; reviewed and edited manuscripts), Dr. Mario Tenuta (provided lab resources and experimental methods), Dr. Jonathan Adams (reviewed and edited manuscripts), Dr. Theresa Adesanya (reviewed and edited manuscripts), and Dr. Afua Mante (reviewed and edited manuscripts), and Fernanda Gouvea Pereira (assisted with developing experimental methods).

1. INTRODUCTION

1.1 The oil and natural gas industry

The oil and natural gas (ONG) industry is a major source of global energy for both industrial and domestic uses. Since the mid-1950s, the ONG industry has provided more than 40% of the total global energy consumption (International Energy Agency, 2023). The global demand for ONG products is on the rise and is not expected to abate soon despite the shift from fossil energy sources to renewable energy sources, such as solar and hydropower, to reduce greenhouse gas emissions (Gong, 2020). Greenhouse gas emissions from natural gas are 45-65% lower than those from coal (Hartmann et al., 2021). As a result, global industrial and domestic consumption of natural gas is projected to grow as countries seek to lower their greenhouse gas emissions by substituting coal with natural gas for heating and power generation (Hartmann et al., 2021). For oil, the increase in demand will be driven more by the demand for petrochemicals and less by industrial fuel needs (International Energy Agency, 2023).

Globally, Canada ranks fourth and fifth in oil and natural gas production by volume, respectively (Natural Resources Canada, 2024). The ONG industry contributes 5% to Canada's GDP (Statistics Canada, 2021) and employs approximately 740,000 people, providing an estimated \$20 billion per year in taxes, royalties, and fees to provincial governments (Canada Energy Regulator, 2023a). Canada's ONG operations are active in 12 of the 13 provinces and territories, with the largest reserves in the provinces of Alberta, Saskatchewan, Newfoundland and Labrador, and British Columbia (Canada Energy Regulator (CER), 2023a). Due to these huge ONG reserves, Canada is expected to grow and develop its ONG industry to meet the increasing local and international demand (Canada's Oil & Natural gas Producers (CAPP), 2023).

Pipelines are the preferred mode of transport of ONG products locally and across international borders owing to their relative safety, convenience, and cost-effectiveness compared to rail and road transportation (Belvederesi et al., 2018). Canada has approximately 840,000 km of pipeline infrastructure (Canada Energy Regulator, 2023b). The increase in global energy demand is ultimately driving an increase in ONG exploration, hence, the development of associated infrastructure such as pipelines for improved handling capacity. For instance, in British Columbia alone, approximately 2,981 km of pipelines were constructed in 2019, a 6% increase from the 2018 pipeline inventory (Canada Energy Regulator, 2023b).

1.2 Pipeline right-of-way

A right-of-way (ROW) is a strip of land where a pipeline company has legal access to install, operate, inspect, maintain, and repair a pipeline. The right-of-way is typically acquired through agreement, easement, or lease with the landowner. As such, the ROW becomes the area impacted by pipeline installation activities (Figure 1.1). The ROW can be divided into three distinct regions: the trench area, the working area, and the soil piling area (Desserud et al., 2010). ROW width varies with the size of the pipeline being installed, the installation method, and the type of equipment used. For example, ROW widths of 18.3 m (Culley et al., 1982), 23 m (Soon et al., 2000a), 30 m (Desserud et al., 2010), and 50 m (Brehm and Culman, 2023) have been reported in previous studies. Although pipeline construction impacts the entire ROW, the extent of land disturbance varies among the three ROW zones and decreases in the order trench > working area > soil piling area (Soon et al., 2000b; Desserud et al., 2010; Shi et al., 2015). To minimize the ecological impacts of pipelines, new pipelines are often sited on existing ROWs (Neville 2003). This practice is particularly important on cropland, where the cumulative size of land impacted by underground pipeline installation could be detrimental to farm productivity.

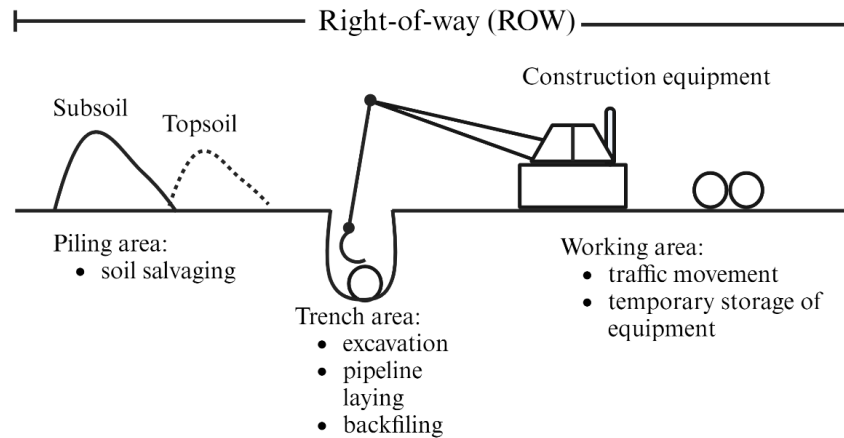


Figure 1. 1 Typical zones of an underground pipeline right-of-way (ROW).

Many studies attribute pipeline construction impacts on soil and vegetation ecosystems to movement of heavy vehicles, topsoil stripping, and horizon mixing during excavation, soil piling, and trench backfilling (e.g., Soon et al., 2000a; Shi et al., 2015; Gasch et al., 2016). Traditionally, ROW reclamation guidelines require excavated soil to be separated by horizons (i.e., topsoil and subsoil) during construction, a practice known as “double-lifting” (Naeth et al., 1987; Nielsen et al., 1990; Desserud et al., 2010). Double lifts are thought to decrease the rates of soil mixing between soil horizons which typically differ in texture, porosity, organic matter content, and soil chemistry (Brehm and Culman, 2022).

Where possible, pipeline ROWs avoid prime agricultural land, wetlands, and any potentially unstable areas (Bayramov et al., 2012). However, in certain situations, these areas are unavoidable. For example, for logistical reasons, ROWs sometimes traverse prime agricultural land in the Agricultural Land Reserve (ALR) of British Columbia, Canada (Figure 1.2). The ALR is a zone in British Columbia where agriculture is recognized as the top priority land use and non-agricultural land uses are restricted (B.C. Minister of Agriculture’s Advisory Committee, 2018). Because of the importance of agriculture and ONG to the province’s economy, the Agricultural

Land Commission (ALC) allows ONG infrastructure on the ALR based on the commitment from the ONG industry that it would restore the land back to the agricultural productivity that existed prior to pipeline construction. Thus, the ALC views ONG activities on cropland as temporary, albeit long-term.



Figure 1.2 A pipeline ROW traversing cropland (swathed canola field) in northeast British Columbia, Canada (September 2022).

1.3 Impacts of pipeline construction on soil and crop productivity

1.3.1 Impacts on soil chemical properties

Pipeline construction methods that separate soil horizons such as the “double-lifting” technique minimizes but does not eliminate the mixing of soil horizons during pipeline construction (Desserud et al., 2010). Admixtures between the fertile topsoil and the less fertile subsoil have the greatest impact on soil chemical attributes (Gasch et al., 2014; Shi et al., 2014; Xiao et al., 2014). Mixing soil horizons depreciates the nutrient status of the topsoil, thus reducing its productivity following reclamation (Olson and Doherty, 2012; Gasch et al., 2016). In addition, stockpiled soil is vulnerable to nutrient losses through leaching and soil erosion if left open to precipitation for extended periods (Desserud et al., 2010; Shi et al., 2014). The greatest changes in

soil chemical properties due to pipeline construction occur in the top 20 cm, with little or no change in chemical properties below the 50 cm depth (Soon et al., 2000b). Several studies showed lower concentrations of organic matter (SOM), total nitrogen (TN), available phosphorus (AP), soluble sulphate (S), and exchangeable calcium (Ca), and higher pH and electrical conductivity (EC) on ROWs relative to adjacent off-ROWs (e.g., Soon et al., 2000b; Shi et al., 2015; Brehm and Culman, 2023). A recent meta-analysis of 26 studies showed that an increase in topsoil pH is a widespread problem on reclaimed ROWs (Birkhimer et al., 2023). Changes in soil pH could have adverse effects on the availability of some nutrients, thus negatively impacting crop nutrition. For example, soil pH above 7 could reduce P solubility, rendering the soil P deficient.

Since reclaimed ROWs are typically lower in plant available nutrients than off-ROWs, fertilizers are usually applied to aid plant establishment and growth during ROW revegetation programs (Sheoran et al., 2010). Crops on reclaimed ROWs sometimes receive higher fertilizer rates than recommended to optimize crop yields. However, the response of crop yields to fertilizer application is variable. A study in eastern Ontario and Quebec evaluated the effect of fertilizer, fertilizer-plus-manure, and season of pipeline installation on corn yield (Neilsen et al., 1990). In the study, fertilizer and fertilizer-plus-manure treatments increased corn yields on the ROWs. However, while fertilizer-manure treatments increased yield to levels of the off-ROW sites, yield gains from fertilizer application were not sufficient to compensate for yield losses due to pipeline installation. Fertilizer and manure applications have been used successfully to hasten the recovery of land disturbed by other anthropogenic activities, such as mining. For example, faster vegetation restoration has been reported with fertilizer application relative to unfertilized treatments (e.g., Rowland et al., 2009; Howell et al., 2016). The authors concluded that repeated fertilizer

application on reclaimed land can be used to create nutrient profiles similar to those of undisturbed benchmark soils.

1.3.2 Impacts on soil physical properties

The impacts of ONG pipeline construction on soil physical properties are largely due to soil compaction induced by the movement of construction vehicles and the physical presence of the pipeline and its contents in the soil environment. Soil compaction reduces the size and number of meso- and macropores and also disrupts soil pore continuity (Weil and Brady, 2017). As a result, bulk density, soil strength, and penetration resistance (PR) tend to increase on the pipeline ROWs relative to adjacent off-ROWs (Tekeste et al., 2020), while steady-state infiltration rates, hydraulic conductivity, water retention, and aggregate stability are greatly reduced on the pipeline ROWs (Culley et al., 1982; Soon et al., 2000a; Muitire et al., 2024).

In crop production systems, soil compaction is highly undesirable. It impedes crop root growth and development due to increased PR and soil strength, poor soil aeration, slow water and nutrient movement, and the buildup of toxic gases (Weil and Brady, 2017). Inhibited crop rooting reduces water and nutrient uptake and crop anchorage, thus adversely impacting crop productivity (Weil and Brady, 2017; Tekeste et al., 2020). Negative linear correlations of crop root growth and soil PR have been reported for oats (*Avena sativa*) (Ehlers et al., 1983), corn (*Zea mays*) (He et al., 2017), and soybean (*Glycine max*) (Moraes et al., 2020), with yield reductions of 9-50% reported for corn and soybean on pipeline ROWs relative to adjacent off-ROWs (Tekeste et al., 2020). Ebrahimi et al. (2022) reported a reduction in plant available water retention capacity of up to 15% on ROWs relative to off-ROWs.

Soil compaction takes decades to naturally ameliorate through plant root activity and other biological processes (Batey, 2009). In pipeline construction, strategies that minimize negative

impacts of compaction on soil properties have been researched and developed. These include the construction of underground pipelines in winter when the soil is frozen (Neilsen et al., 1990), avoiding pipeline installation during the wet seasons (Soon et al., 2000a), and the use of relatively lighter construction vehicles (Tekeste et al., 2019). Minimizing the impacts of pipeline construction on soil properties would also reduce the time required to restore reclaimed ROWs to pre-disturbance productivity.

The physical presence of the ONG pipelines and their contents creates a micro-environment around the pipeline characterized by elevated soil temperature (Naeth et al., 1993). Shi et al. (2015) reported a 1 to 2 °C increase in soil temperature above an operating underground pipeline on cropland. Elevated soil temperatures on the ROWs have also been reported by Halmová et al. (2017). Elevated soil temperatures could have detrimental impacts on other soil properties, such as reducing soil moisture content due to increased evapotranspiration (Gasch et al., 2016; Halmová et al., 2017), which could adversely impact crop yields, particularly in dry environments or during dry seasons.

1.3.3 Impacts on soil biological properties

Soil microorganisms are mostly concentrated in the topsoil, in the Ah horizon, and close to plant roots in the rhizosphere relative to the rest of the bulk soil (Basiliko et al., 2021). As such, the stripping of topsoil, mixing of topsoil and subsoil, and uprooting of vegetation during pipeline construction could potentially impact soil microbial population structure and functionality. Soil microorganisms carry out and facilitate soil processes such as nutrient cycling, organic matter decomposition, soil energy transfer, mineralization of organic nutrients, stabilization of organic matter, aggregate formation, and promoting plant health by suppressing plant-pathogenic microbes and phytophagous fauna (Doran and Zeiss, 2000; Dangi et al., 2012; Hamidović et al., 2020).

Therefore, any disturbance of microbial communities can have cascading effects on overall soil ecosystem functionality and ultimately affect soil productivity. However, dynamics of soil microbial communities on underground ONG pipeline ROWs have not received much attention from researchers compared to soil physical and chemical properties (Brehm and Culman, 2023).

Few studies that have characterized microbial communities on pipeline ROWs have employed non-nucleic acid-based techniques, which generalize across microbial communities and are therefore not sensitive to changes in microbial community structure. As such, conflicting results on the impacts of underground pipeline construction on soil microbial communities have been reported. For instance, Soon et al. (2000b) measured microbial biomass carbon on ROWs to evaluate microbial communities in Alberta, Canada and showed that, on average, microbial biomass carbon was not adversely impacted by pipeline installation. On the other hand, phospholipid fatty acid analysis was used in a study conducted following pipeline ROW reclamation on a sagebrush steppe in Wyoming, USA (Gasch et al., 2016). The study showed a decrease in total microbial abundance on one year-old ROWs compared to adjacent off-ROWs, whereas total microbial abundance on 5-, 36-, and 55-yr ROWs did not differ significantly from that on the reference sites (Gasch et al., 2016). The authors attributed the lower microbial abundance on the one-year ROW to soil horizon mixing and the temporal increase in microbial abundance to natural recovery of the ROWs.

Soil microbial community studies could be scaled by using nucleic acid analysis techniques such as 16S rDNA (for bacterial genome) and internal transcribed spacer (ITS) (for fungal genome) gene sequencing. These techniques generate huge datasets, which could be used to extensively characterize microbial communities at phylum, taxon, and species levels. Richness and abundance indices such as the Chao1, abundance-based coverage estimator (ACE), Shannon, and

Simpson could also be employed on the data to evaluate microbial community structures (Wei et al., 2018). Microbial richness refers to the number of microbial species or taxa in the soil sample, while abundance refers to the total number or biomass of microorganisms within a community. Chao1 and ACE are abundance-based richness estimator indices, while Shannon (Shannon-Wiener index) and Simpson are diversity indices. Diversity indices provide information on the richness and evenness of microbial communities (Wang et al., 2019). These indices have been used successfully to evaluate the restoration of reclaimed mining sites (e.g., Ezeokoli et al., 2020; Lu et al., 2018; Rosenfeld et al., 2018) and the effectiveness of different land management strategies (e.g., Wang et al., 2019; Wei et al., 2018). Therefore, these indices could also be effective in evaluating the restoration success of underground pipeline ROWs on cropland, hence their use in the present study.

1.3.4 Soil health on reclaimed ROWs

Soil health is the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS, 2016). The term provides a comprehensive overview of the state of the soil because it acknowledges that no one group of soil attributes can sufficiently describe the capacity of the soil to sustain life (Karlen et al., 2019; Van Eerd et al., 2021). However, soil health assessments do not look at all soil attributes in their totality, but only key indicators that have been proven to be most sensitive to changes in soil management (Doran, 2002). There are ongoing efforts to come up with a minimum suite of indicators that are relevant, sensitive to changes in the soil ecosystem, cost-effective, easy to measure, accurate, correlated to soil functions, and informative (Bagnall et al., 2023; Liptzin et al., 2023). More recently, the Soil Health Institute (USA) published and recommended a suite of three soil health indicators (soil aggregate stability, carbon mineralization potential, and soil organic carbon concentration) after

evaluating over 30 soil health indicators at 124 long-term agricultural research sites across north America (Bagnall et al., 2023). These soil health indicators are sensitive to changes in land use, relatively easy to measure, and are correlated with soil ecosystem services (Bagnall et al., 2023).

Few studies (e.g., Soon et al., 2000b; Schindelbeck and van Es, 2012) have evaluated soil health on reclaimed ROWs on cropland. This is despite evidence from several studies pointing to soil health indicators such as permanganate-oxidizable C (POXC), autoclaved citrate-extractable (ACE) protein, aggregate stability, and soil respiration as sensitive to changes in land use and, therefore, having the potential to serve as valuable measures of directional change in soil health (e.g., Liptzin et al., 2022; Liptzin et al., 2023; Bagnall et al., 2023). However, since studies have shown the negative effects of pipeline installation on soil attributes, it could be hypothesized that soil health would be lower on reclaimed ROWs than on off-ROWs and would improve with increasing time since reclamation (TSR). As such, sensitive soil health indicators could, therefore, be used to examine and evaluate the recovery of reclaimed ROWs.

1.3.5 Impacts on crop productivity

The continued expansion of pipeline networks is increasingly threatening the size and quality of farmland, necessitating the accurate quantification of the impacts of oil and natural gas pipeline infrastructure construction on soil productivity. Several studies across the world showed lower crop productivity on reclaimed ROWs than on the off-ROW on the same field despite the application of sufficient fertilizer nutrients (e.g., Soon et al., 2000b; Shi et al., 2015; Brehm and Culman, 2023). Yield losses due to pipeline construction depend on several factors, such as baseline soil properties (Shi et al., 2015), crop type (Xiao et al., 2016; Brehm and Culman, 2023), timing of pipeline construction (Neilsen et al., 1990), and installation method (Tekeste et al., 2020). Crop yield losses ranging from 13-50% have been reported on reclaimed ROWs. For

instance, in Ontario, Canada, Culley et al. (1982) reported yield reductions of 50% for corn grain yield 2 yr after ROW reclamation and 29% for soybean yield 1 yr following ROW reclamation on poorly drained, fine-textured clays. In Iowa, USA, corn and soybean yields declined by an average of 27% on the ROW relative to the off-ROW (Ebrahimi et al., 2022). In another study, Brehm and Culman (2023) reported yield reductions of 24% for corn and up to 13% for soybean averaged across varying soil types 5 yr after reclamation of ROWs in Ohio, USA. In most of these studies, crop yield losses resulting from pipeline construction were associated with a reduction in plant physiological attributes such as a decrease in plant height, stem size, cob length, cob size, leaf chlorophyll content, and delayed silking on the ROW relative to the off-ROW (Shi et al., 2015; Neilsen et al., 1990). Yield losses observed on the ROWs in these studies were attributed to changes in soil properties, particularly reduced plant available nutrients and increased soil pH and compaction, due to pipeline construction activities.

1.4 Effect of time on recovery of ROWs

Few studies have evaluated the effect of time elapsed since reclamation (TSR) on soil and vegetation recovery on ROWs on cropland. To the best of our knowledge, none of the studies have evaluated soil properties and crop productivity on ROWs of TSR > 10 yr. Nonetheless, it is generally acknowledged that the recovery of soil and vegetation attributes on reclaimed ROWs is a gradual process that can take several years (e.g., Soon et al., 2000b; Shi et al., 2014; Brehm and Culman, 2023). A reclaimed ROW could be considered to be on the recovery trajectory if its productivity is converging towards pre-disturbance capability or productivity on adjacent undisturbed sites. In Canada, reclaimed sites are considered fully recovered if soil properties and vegetation attribute measurements are $\geq 80\%$ of values on undisturbed sites (Environment and Sustainable Resource Development, 2013). Reference values could either be baseline properties

(pre-disturbance) or properties on adjacent undisturbed sites. These regulations apply to all ecosystems disturbed by industrial activities in Canada.

Although an increasing number of studies (e.g., Soon et al., 2000a; Shi et al., 2015; Brehm and Culman, 2023) have shown that soil and vegetation attributes on reclaimed ROWs improve with increasing TSR, albeit slowly, the time required for complete recovery is not clear-cut. In wetlands in southeast Wisconsin, USA, it took 8 yr post-reclamation for bulk density, soil moisture, and vegetation cover on the pipeline ROW to fully recover to pre-disturbance conditions (Olson and Doherty, 2012). In the agricultural provinces of Gansu and Shaanxi in China, reclaimed ROWs fully recovered 6 yr post-reclamation, as shown by a soil restoration index of 98.6% on the trench area and 100% on the working area (Shi et al., 2014). In other studies in Canada (Culley et al., 1982) and Ohio, USA (Brehm and Culman, 2023), crop yield losses on reclaimed ROWs were still evident 5 yr post-reclamation. Individual soil properties recover to pre-disturbance levels at varying rates. For example, it took 3 yr following ROW reclamation for EC, TN, and soluble sulphates to recover to pre-disturbance levels while soil pH, available P, and K took longer (Soon et al., 2000b).

Variability in the recovery periods of reclaimed ROWs could be due to varying site characteristics, pipeline construction techniques, reclamation methods, or post-reclamation land management strategies. Recovery of ROWs tends to be faster in dry climates than in wet climates (Soon et al., 2000b). Pipeline ROW recovery took more than 10 yr in the wet climate of eastern Canada while it took 3 yr in drier western Canada (Soon et al., 2000b). Soon et al. (2000b) concluded that there was less deterioration of soil attributes, particularly soil physical properties, in dry climates compared to the wet climates. Neilsen et al. (1990) observed that impacts of ONG pipeline installation were less on winter-installed pipelines than on autumn-installed pipelines.

Desserud et al. (2010) also observed faster establishment of grasses on minimally-disturbed sites where pipelines were constructed in late fall or winter than on spring- and summer-installed pipeline ROWs. The authors attributed the differences in recovery rate to varying soil moisture contents during the seasons. Soils are frozen in winter and, therefore, drier compared to other seasons, resulting in less detrimental impacts, particularly soil compaction.

The general improvement in soil and vegetation properties on reclaimed ROWs with increasing TSR could be attributed to the influence and interaction of natural soil recovery processes and land management practices (e.g., Soon et al., 2000b; Gasch et al., 2014; Shi et al., 2015). On cropland, the recovery of reclaimed ROWs could be influenced by agronomic practices. For example, minimum soil disturbance, crop rotation, and retention of crop residues are known to help build soil properties (Bagnall et al., 2023; Liptzin et al., 2023). These practices can slowly build soil carbon pools, which improve microbial communities and functioning, nutrient cycling, and overall soil functioning (Stetson et al., 2018; Williams et al., 2020; Van Eerd et al., 2021), thus enhancing the recovery of reclaimed ROWs. Bagnall et al. (2023) noted a significant increase in soil organic carbon and ACE protein under reduced tillage, cover cropping, and residue retention in a study conducted on 124 agricultural research sites across North America. The addition of organic amendments either during reclamation or as a land management strategy post-reclamation has been shown to hasten the recovery of disturbed land by increasing soil organic carbon concentration (Larney and Angers, 2012). Although research has shown that reclaimed ROWs can successfully revert to pre-disturbance capabilities with increasing TSR, landowners, policymakers, and environmentalists are still grappling with the question on how long such recovery will take.

1.5 Thesis objectives

There is a dearth of information on the influence of time since reclamation on the recovery of soil properties and crop productivity on underground natural gas pipeline ROWs on cropland. Previous studies have focused mostly on reclaimed ROWs in non-agricultural ecosystems. Of the few studies focusing on cropland, even fewer evaluated soil microbial communities and soil health. Therefore, the overall objective of this thesis was to quantify the impact of pipeline construction on soil properties and crop productivity and the changes thereof with increasing time since reclamation. Specific objectives of this study were to (i) determine the effects of underground pipeline construction and time since reclamation on soil health and crop yield and quality (protein content) (Chapter 2); (ii) evaluate the changes in soil physical properties with increasing time since reclamation (Chapter 3); (iii) evaluate the structure of soil bacterial and fungal communities on reclaimed ROWs and their changes with increasing time since reclamation (Chapter 4); and (iii) determine the response to fertilizer application rates of canola and wheat grown in soils collected from reclaimed natural gas ROWs (Chapter 5).

1.6 Thesis outline

The outline of this thesis follows the thesis guidelines of the Department of Soil Science, University of Manitoba. Chapters 2 through 5 were prepared in manuscript format. **Chapter 1** is an introductory chapter that provides information on the impacts of underground pipeline construction activities on the soil and vegetation ecosystems. In addition, the chapter reviews studies conducted on reclaimed ROWs across the world, narrowing down to studies conducted in Canada. The main objective of the chapter was to identify the existing information gaps on the

subject area. The information gaps are the foundation on which Chapters 2 through 5 are based. The chapter further outlines the scope of the thesis and its objectives and general structure.

Basing on the information gaps identified in Chapter 1, **Chapter 2** investigates temporal trends in soil health and productivity on reclaimed natural gas pipeline rights-of-way on cropland. This chapter uniquely employs soil health indicators and crop yield and quality to evaluate the impacts of pipeline construction on soil productivity and to determine the influence of TSR on the recovery of productivity on cropland disturbed by pipeline construction.

Expanding on the information presented in Chapter 2, **Chapter 3** explores temporal changes in soil physical properties on the reclaimed ROWs investigated in Chapter 2. This chapter also links soil physical properties to crop yield results presented in Chapter 2. This linkage helps to explain how pipeline construction activities affect crop yield through impacts on soil physical properties. The chapter also addresses the underlying factors influencing water transport on reclaimed ROWs by exploring soil pore indices on the ROWs.

The literature review summarized in the introductory section of Chapter 2 revealed that soil biological properties on reclaimed ROWs have received less attention from researchers relative to soil physicochemical properties. As such, **Chapter 4** explores the dynamics of microbial communities on reclaimed ROWs on cropland. The novelty of this chapter is that it employs nucleic acid techniques (16S rRNA and ITS gene sequencing) to investigate the diversity and abundance of soil microorganisms on reclaimed ROWs of varying ages. Results reported in this chapter are linked to the crop yield results presented in Chapter 2, which also helps to explain the observed trends in crop yields with increasing TSR.

The results presented in Chapters 2, 3, and 4 suggested negative impacts of active natural gas pipelines on the productivity of reclaimed ROWs. As such, a growth room bioassay was

conducted to determine the productivity of reclaimed ROW soils in the absence of an operating natural gas pipeline (**Chapter 5**). This was done by growing crops under controlled environment conditions using soils collected from the reclaimed ROWs investigated in Chapters 2, 3, and 4. Chapter 5 also evaluated the possibility of improving crop yields on reclaimed ROWs by increasing fertilizer application rates.

An overall synthesis of findings reported in the research chapters is presented in **Chapter 6**. This chapter also discusses the implications of the study, its limitations, and recommendations for future research.

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2. TEMPORAL TRENDS IN SOIL HEALTH AND PRODUCTIVITY ON RECLAIMED NATURAL GAS PIPELINE RIGHTS-OF-WAY ON CROPLAND

2.1 Abstract

The construction of underground pipelines negatively impact soil productivity in various ecosystems. However, the temporal progression in the recovery of soil productivity following reclamation of cropland impacted by natural gas pipeline rights-of-way construction remains unclear. This study examined temporal, post-reclamation changes in selected soil health indicators and productivity on reclaimed underground natural gas pipeline rights-of-way (ROWs) on cropland. Soil and crop (canola, wheat, barley, oats, and field peas) samples were collected from ROWs ranging in time elapsed since reclamation (TSR) from 6 to 12 yr and from adjacent undisturbed locations (off-ROW) in the same field. The soil samples were analyzed for soil health indicators (permanganate-oxidizable C (POXC), soil respiration, and autoclaved citrate-extractable (ACE) protein) and selected chemical properties. Crop samples were assessed for grain and total biomass yields as well as grain protein content. Soil organic C on the ROWs was 29% (6-yr ROW) and 33% (9-yr ROW) lower than on the off-ROWs. Soil respiration recovered within 6 yr of ROW reclamation, whereas it took 12 yr for POXC and ACE protein to recover to off-ROW levels. Grain and biomass yields 12 yr post-reclamation were still 42% and 36%, respectively, lower on the ROWs than on the off-ROWs. However, measured soil attributes recovered faster than crop variables, indicating that pipeline construction on cropland has longer-term impacts on crop yields than on the measured soil attributes. These results indicate that, although underground pipeline construction has detrimental impacts on soil and crop attributes, these attributes would slowly recover to pre-construction levels with increasing TSR.

2.2 Introduction

The global demand for oil and natural gas products is expected to continue growing into the foreseeable future (Gong, 2020). To keep up with the increasing demand, oil and natural gas exploration and development is also increasing, posing a growing threat to agriculture and food security as oil and natural gas infrastructure, such as pipelines, at times traverse cropland. Pipeline construction activities include vegetation removal, movement of heavy construction vehicles, soil excavation, soil piling (salvaging), and trench backfilling (Desserud et al., 2010; Gasch et al., 2014). Among these activities, the movement of heavy construction vehicles and soil horizon admixing that occur during excavation, soil piling, and trench backfilling have the greatest impacts on the soil ecosystem, hence productivity (Shi et al., 2015; Gasch et al., 2016). In addition, leakages from operating pipelines can potentially alter soil properties jeopardizing the recovery of the pipeline right-of-way (ROW) after reclamation. For example, crude oil spills on agricultural land significantly reduced microbial biomass carbon and microbial metabolic diversity (Timmerman et al., 2003). The ROW is the entire width and length of the area that is impacted by underground pipeline construction activities. To substantially reduce the size of the pipeline footprint, new pipeline routes usually utilize existing ROWs (Neville, 2002). Regardless, the cumulative cropland area impacted by several overlapping ROWs remains a concern to landowners.

While several studies have highlighted the impacts of pipeline construction on soil and vegetation ecosystems (e.g., Culley et al., 1982; Soon et al., 2000a; Brehm and Culman, 2023), there is a knowledge gap on the influence of time since reclamation (TSR) on the recovery of reclaimed ROWs. Impacts of pipeline construction include reductions in vegetation cover (Bayramov et al., 2012; Naeth et al., 2020), crop productivity (Soon et al., 2000b; Shi et al., 2015),

plant available nutrients (Soon et al., 2000a; Xue et al., 2006), soil moisture retention (Olson and Doherty, 2012), aggregate stability (Brehm and Culman, 2023), and hydraulic conductivity (Soon et al., 2000a; Muitire et al., 2014). On the other hand, soil temperature (Naeth et al., 1993; Shi et al., 2015), penetration resistance (Tekeste et al., 2019), soil pH and electrical conductivity (EC) (Soon et al., 2000a) have been reported to increase on the ROW relative to adjacent undisturbed (off-ROW) locations. Soil excavation during pipeline construction also increase C mineralization which reduces soil carbon stocks (Mason et al., 2011). A study on the recovery of pipeline ROWs in a boreal plain soil showed that most pipeline construction-induced changes to soil chemical properties occurred in the top 0-20 cm soil layer (Soon et al., 2000a). The study showed lower concentrations of soil organic matter (SOM), total nitrogen (TN), available phosphorous (AP), soluble sulphates (S), and exchangeable calcium (Ca), and higher pH and EC on the ROW relative to the off-ROW (Soon et al., 2000a). Other studies conducted on cropland also reported lower nutrient concentration on reclaimed ROWs than on the off-ROW (e.g., Culley et al., 1982; Shi et al., 2015; Brehm and Culman, 2023).

The recovery of soil and vegetation attributes on the ROWs to pre-disturbance levels is a gradual process that can take several years (Shi et al., 2015; Brehm and Culman, 2023). For example, in wetlands in southeast Wisconsin, USA, it took 8 yr for bulk density (BD), soil moisture retention, and vegetation cover on the pipeline ROW to fully recover (Olson & Doherty, 2012). In the agricultural provinces of Gansu and Shaanxi in China, Shi et al. (2014) showed that the soil restoration index (SRI) on the trench area was 98.6% and 100% on the working area 6 yr post reclamation showing that these areas had recovered to the undisturbed state in 6 yr. Variability in the recovery periods of pipeline ROWs is partly a function of baseline soil properties (Soon et al., 2000a), the pipeline installation method (Bayramov et al., 2012), the construction equipment used

(Tekeste et al., 2020), construction timing (Neilsen et al., 1990), and land management post construction (Tekeste et al., 2020). Soil properties also recover to undisturbed state at varying rates. For instance, Soon et al. (2000a) showed that TN and microbial communities recovered within 3 yr, whereas available P and K took longer to recover on the Canadian boreal plains.

Changes to the soil ecosystem manifest in poor above ground vegetation characteristics such as shorter plant heights, delayed silking in corn, and lower grain yield on the ROWs than on the off-ROW (Culley et al., 1982; Shi et al., 2015). For instance, a study in Iowa, USA, showed a reduction in growth rate, plant height, and a 27% yield loss in maize and soybean on the ROW relative to the off-ROW, which was attributed to increased penetration resistance and bulk density and reduced soil moisture retention, macroporosity, and saturated hydraulic conductivity on the ROWs (Ebrahimi et al., 2022).

Similar to soil properties, the impacts of pipeline construction on crop productivity are long-term. For example, in Iowa, USA, corn and soybean yields were still 7.4-23.8% lower on the ROW than on the adjacent off-ROW 5 yr post-reclamation (Brehm and Culman, 2023). In another study, a 28% reduction in cereal yield was observed 10 yr post-reclamation of pipeline ROWs in Alberta, Canada (Culley & Dow, 1988). Yield reductions in these studies were largely attributed to reduced nutrient concentrations due to horizon mixing and increased soil compaction on the ROW relative to the off-ROW.

Underground pipelines have been accommodated on prime agricultural land based on evidence of the eventual recovery of productivity on the ROWs with increasing time after reclamation. However, the long duration of the recovery, hence extended loss of productivity on the ROW, remains a concern to landowners. Although landowners are compensated for the loss in productivity on the ROWs, in some cases, the loss in productivity could outweigh the

compensation because there is a general assumption of recovery of productivity on the ROW within a few years post-reclamation, for example, 5 yr in Iowa, USA (Brehm and Culman, 2023). In addition, disturbed land is considered recovered if soil and vegetation attributes on the reclaimed land meet mandatory reclamation goals (British Columbia Energy Regulator (BCER), 2017). For example, in Canada, soil attribute levels on the reclaimed land must be $\geq 80\%$ of those on adjacent undisturbed sites in order for the site to be certified reclaimed (Environment and Sustainable Resource Development (ESRD), 2013). Although this might be satisfactory to policymakers and environmentalists, a yield reduction of 20% on the ROW relative to the off-ROW could be substantial to producers. In any case, regulatory assessments on reclaimed cropland in western Canada are based on plant height and seedling count (British Columbia Energy Regulator (BCER), 2017), which may not necessarily correlate with crop yield. Therefore, a site regarded as recovered based on the regulatory criteria might not be sufficiently recovered for the farmer necessitating the need for comprehensive studies on the recovery of productivity on reclaimed ROWs.

Most studies on the recovery of vegetation and soil attributes on underground pipeline ROWs have focused on non-agricultural ecosystems and plants such as weeds, wild grasses, shrubs, and trees (e.g., Desserud et al., 2010; Olson and Doherty, 2012; Naeth et al., 2020), with only a handful focusing on crops and farmland (e.g., Neilsen et al., 1990; Soon et al., 2000a; Brehm and Culman, 2023). Of the few, even fewer (e.g., Shi et al., 2015; Brehm and Culman, 2023) have evaluated the effect of time since reclamation on the recovery of productivity on reclaimed ROWs on cropland. In addition, few studies (e.g., Soon et al., 2000b; Schindelbeck and van Es, 2012) have characterized reclaimed pipeline ROWs using soil health indicators. This is despite the findings of several studies which have shown that some soil health indicators, for example permanganate-oxidizable C (POXC), autoclaved citrate-extractable (ACE) protein, and soil

respiration are sensitive to changes in land use and may serve as valuable measures of directional changes in soil health (e.g., Liptzin et al., 2022; Liptzin et al., 2023; Bagnall et al., 2023), hence their suitability for evaluating the recovery of ROWs. Therefore, the objectives of this study were to (1) characterize the dynamics of soil health indicators as a function of time elapsed since completion of ROW reclamation and (2) determine temporal changes in crop yield and quality following reclamation of pipeline ROWs. The study provides an estimate of the minimum time required to restore the productivity of the ROWs to pre-disturbance levels. This information is critical to understanding the effectiveness of current underground pipeline construction approaches and ROW reclamation methods and will assist industry, landowners, and policymakers in formulating strategies for minimizing negative impacts on cropland productivity.

2.3 Materials and Methods

2.3.1 Site description

This study was conducted on three sites near Farmington, British Columbia, Canada (55° 54' 15" N, 120° 30' 10" W; Figure 2.1). The region has typical characteristics of the Canadian prairies dominated by grasslands, with cold winters and short, warm summers. Long-term (1981-2010) seasonal mean temperatures range from -10.1 °C in the winter (November to February) to 13.02 °C in the growing season (May to September), with annual precipitation averaging 444.7 mm (Environment and Climate Change Canada, 2023). The study area has good surface drainage. Site 1 is at an elevation of 757.7 m above mean sea level (amsl) with an average slope of 2.6%, site 2 is at an elevation of 766.3 m amsl with an average slope of 2.3%, and site 3 is at an elevation of 765.4 m amsl with an average slope of 2.5% (Google Earth Pro, 2024). The dominant soils are

acid to neutral pH and described as Orthic Grey Luvisols (Boralfs in the U.S. Taxonomy) (B.C. Soil Information Finder Tool, 2018).

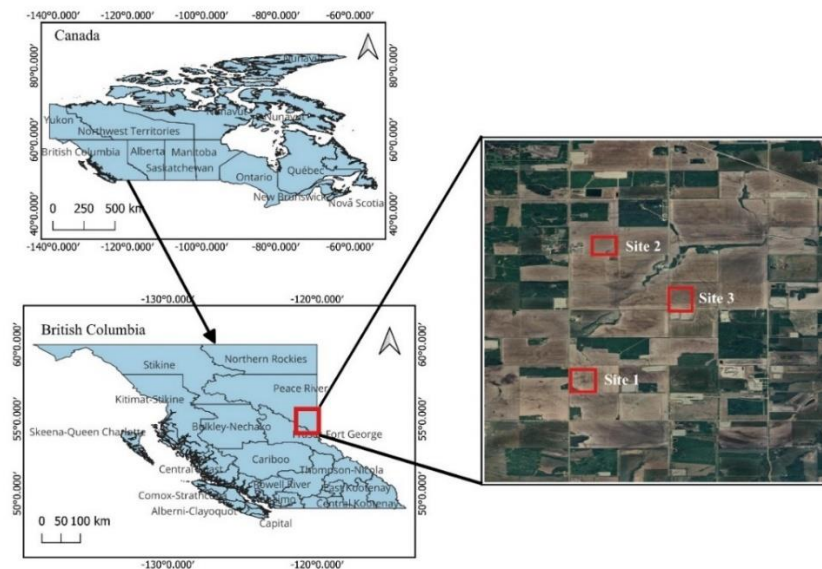


Figure 2. 1 Location of the study sites in northeastern British Columbia, Canada.

Because of its favorable climatic and soil characteristics (Canada Land Inventory Classes 2-4) (B.C. Soils Information Finder Tool, 2018), the region falls under British Columbia's Agricultural Land Reserve (ALR) (B.C. Agricultural Land Commission, 2022) and accounts for one third of the ALR. The ALR (~ 4.7 million ha, or 4.9% of British Columbia's land area) is the provincial zone established to protect agricultural land and in which agriculture is the priority land use, with non-agricultural uses restricted (B.C. Agricultural Land Commission Act, 2019). Canada Land Inventory Classes 1-4 lands are generally considered the key agricultural lands of British Columbia and are included within the province's ALR, although forage lands (Class 5) and spring and fall grazing lands (Class 6) are also included. Agricultural activities in the study area include livestock and crop production, with the major crops being canola (*Brassica rapa* L.), spring wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), field pea (*Pisum sativum* L.), and forages. Farmers participating in this study practice crop rotations and crop

residue retention with harrowing to even out crop stubbles with periodic sub-soiling. They also practice minimum tillage using zero-till drills with hoe-type openers for seeding. The region is also rich in oil and natural gas resources. Accommodation of the oil and gas activities on the ALR was based on the understanding that agricultural land disturbed by activities such as underground pipeline construction would be restored, through reclamation, back to the productivity that existed prior to the disturbance (B.C. Agricultural Land Commission, 2012).

2.3.2 Description of the pipeline rights-of-way

Soil and crop samples were collected from a total of 10 ROWs across three sites and three growing seasons (2020, 2021, and 2022) (Table S2.1). The ROWs were identified using pipeline markers located at the edges of the field in combination with ROW maps from the British Columbia Energy Regulator and landowners' records of the fields and the pipelines. The pipeline ROWs were distinguished by the number of years elapsed since completion of reclamation (time since reclamation, TSR), which translates to the post-reclamation age of the ROW. Since measurements from each ROW were taken over a three-year period, we used the average TSR of each ROW to account for the increasing age over the sampling period. Thus, the TSRs of 6, 9, and 12 yr reported herein represent pipeline age ranges of 5-7, 8-10, and 11-13 yr. All pipelines were installed at a depth of 1.2-2 m. Particle size analysis showed that soils on the ROWs contained 27-41% sand, 40-52% silt, and 17-27% clay in the 0-15 cm layer and 21-31% sand, 26-48% silt, and 31-41% clay in the 15-60 cm layer. By comparison, particle size distribution on the off-ROW consisted of 25-31% sand, 46-54% silt, and 21-27% clay in the 0-15 cm layer and 21-29% sand, 38-50% silt, and 21-41% clay in the 15-60 cm layer.

2.3.3 Sampling design

The natural gas pipeline ROWs of varying TSR (i.e., 6, 9, and 12 yr) plus the undisturbed locations adjacent to each pipeline (off-ROW) were the treatments. Soil and crop samples were collected from four locations (4 m² each) on each ROW and on the off-ROW (Figure 2.2). The sampling locations were 25 m apart, allowing us to capture the field variability on the ROW and the off-ROW. The sampling locations were georeferenced during the first sampling event to facilitate sampling from the same locations in the subsequent years. The off-ROW sampling locations were approximately 50 m away from the edge of the pipeline ROW (or corridor) on the same field. Pipeline construction impacts on soil and vegetation ecosystems typically do not extend beyond 30 m from the ROW trench area (Desserud et al., 2010). Consequently, samples collected from 50 m away sufficiently represent the undisturbed parts of the field (off-ROW).

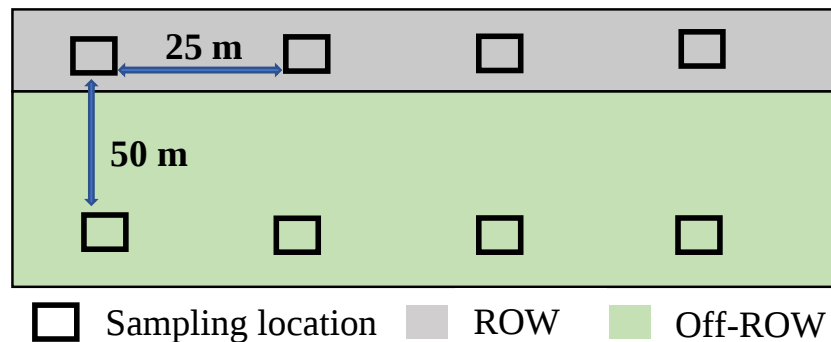


Figure 2.2 Field sampling design.

2.3.4 Crop and soil sampling and processing

Farmers in the study area practice crop rotations (oil seed – cereal – legume). As such, in this three-year study, the crops sampled from the three study sites were wheat, oat, field pea, canola, and barley. During each sampling year, crop samples were collected by cutting plants

approximately 2 cm from the ground using sickles, except for canola, which was sampled from the swathed (harvested and left on the field in rows to dry) crop. Crop samples were packed into clean cloth bags and stored under dry conditions. The samples were transported to the Soil Science laboratory at the University of Manitoba, where they were dried in a drying room at 60 °C for 72 h, followed by weighing to determine total above-ground dry biomass, threshing to separate grain from straw, and weighing of the grain for yield determination. Grain and straw samples were separately ground and passed through a 2-mm sieve. Soil samples were collected from the 0-15 cm (four cores) and the 15-60 cm (two cores) layers in the harvested locations using a Dutch auger. The cores from each depth and location were composited, thoroughly mixed, and subsampled (~ 500 g). The subsamples were dried at 60 °C for 5 d and ground to pass through a 2-mm sieve before analysis for soil health indicators and chemical properties.

2.3.5 Soil health indicators

Soil samples from the 0-15 cm layer were analyzed for soil heterotrophic respiration (24-h CO₂-C), permanganate-oxidizable carbon (POXC), and autoclaved citrate-extractable (ACE) protein at Agvise Laboratories, Northwood, ND, USA. These three soil health indicators are among the indicators most sensitive to changes in land use (e.g., Liptzin et al., 2022; Liptzin et al., 2023; Bagnall et al., 2023) and are therefore suitable for assessing the short- to long-term recovery of reclaimed underground ROWs.

Soil respiration estimates soil biological activity by measuring CO₂ evolution, thus the capacity of the soil to cycle nutrients (C and N Mineralization) (Zibilske, 1994; Franzluebbers et al., 2000). Soil respiration was determined by the Solvita[®] method, which uses a color-coded gel probe to estimate the 24-h CO₂-C burst following rewetting of air-dry soil (Woods End Research, 2016). In this procedure, air-dried soil (40 g) was weighed into a 50-mL perforated beaker with a

glass microfiber filter paper at the bottom to allow the soil to reach field moisture capacity through capillary action (Haney and Haney, 2010). The beaker was then placed in a 200-mL glass jar along with a pH-sensitive Solvita® gel paddle and 12 mL of deionized water. The jars were sealed with a screw-top lid and incubated at 25 °C for 24 h. The amount of CO₂ respired during the 24-h period was determined with a Solvita® digital-color reader (Haney et al., 2018).

Permanganate-oxidizable C was developed to quantify labile C pools (Weil et al., 2003). However, a recent study (Woodings and Margenot, 2023) showed that POXC does not represent labile C fractions because permanganate (MnO₄⁻) can oxidize lignin a recalcitrant form of C as well. Nonetheless, POXC is a sensitive soil health indicator that can be used to evaluate soil C dynamics (Weil et al., 2003). In this study, POXC was determined using the method described by Weil et al. (2003). Briefly, 2.5 g of air-dried soil samples were shaken with 2.0 mL of 0.02 *M* KMnO₄ and 18 mL water for 2 min, allowed to settle for 10 min, and then 0.5 mL of the supernatant was diluted with 49.5 mL distilled water. The absorbance of the solution was then measured with a spectrophotometer at 550 nm.

Autoclaved citrate-extractable protein is a measure of organically bound N in SOM that is easily mineralized by soil microbes (bioavailable N) and therefore provides an indication of long-term N availability to crops (Hurisso et al., 2018; Williams et al., 2020). ACE protein was determined according to Hurisso et al. (2018). Briefly, 8 mL of 0.02 mol L⁻¹ sodium citrate (pH 7.0) was added to 1.0 g soil, thoroughly mixed, autoclaved for 30 min at 121 °C and 15 psi, cooled, centrifuged (3100 × *g*) for 15 min. The supernatants were decanted and ACE protein was estimated by comparing the absorbance (590 nm) values of the extracts to standards.

2.3.6 Soil chemical attributes

Electrical conductivity (EC) and soil pH were measured using an EC meter (Accumet® Basic, AB30) and a pH meter (Accumet® Basic, AB15), respectively, in a 1:2 soil to deionized water paste. All other soil chemical attributes were measured at Agvise Laboratories, Northwood, ND, USA. Nitrate- (NO_3^-) N was measured colorimetrically following reduction with copperized cadmium (Gelderman & Beegle, 2015). Available P (Olsen P) was determined by the ascorbic acid-ammonium molybdate method following extraction (1:10 soil to solution ratio) with 0.5 M sodium bicarbonate at a pH of 8.5 (Frank et al., 2015). Sulfate S was extracted with 0.12 M KCl and determined by turbidimetry (Franzen, 2015). Cation exchange capacity (CEC) was obtained by summing the concentrations of these four cations in ammonium acetate extracts (pH 7). Soil organic matter (SOM) was measured using the loss on ignition (360 °C) method (Combs & Nathan, 2015), a determination method used at Agvise Laboratories. Total carbon (TC) was determined using the dry combustion method (Nelson and Sommers, 1996). Inorganic carbon (IC) was estimated by pressure calcimetry after reacting the soil with 15% HCl (Loeppert and Suarez, 1996). Soil organic carbon (SOC) was calculated as the difference between TC and IC (Nelson & Sommers, 1996).

2.3.7 Plant tissue analysis

Total N concentration was determined in above-ground biomass by the Dumas combustion method (Jones and Case, 1990) while P concentration in the above-ground biomass was determined by spectrophotometry after digestion with 2% acetic acid (Raun et al., 1987; Frank et al., 2015). Nitrogen and P uptake were calculated for each crop as the sum of the products of straw

or grain yield and the corresponding tissue nutrient concentrations. Grain protein content was determined by multiplying grain N content by 5.7 (Tkachuk, 1969).

2.3.8 Crop yield

The yield data collected for this study included five crops (canola, wheat, barley, oat, and field pea). To standardize crop yield across the different crops, sites, and growing seasons, total biomass and grain yield were converted to relative crop yield (RY) using Eq. [2.1]:

$$RY = \frac{Yield_{ROWi}}{Yield_{OFFi}} \quad [2.1]$$

where $Yield_{ROWi}$ is the yield in the i th ROW block, and $Yield_{OFFi}$ is the yield in the corresponding i th off-ROW block.

2.3.9 Statistical analysis

Analysis of variance was conducted on all data using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS[®] OnDemand for Academics (SAS Institute Inc, 2024). Probability distributions used in the analysis were normal for relative grain and biomass yield, nutrient concentration, nutrient uptake, soil pH, EC, and CEC data, beta for SOM data, and lognormal for CO₂-C, POXC, ACE protein, NO₃⁻-N, Olsen P, Ca, and SO₄-S data. Time since reclamation (TSR) was modelled as a fixed effect, while site, block nested within site, and year of sampling were random effects. The Tukey multiple comparison procedure was used for all pairwise comparisons. Effects were considered significant at $\alpha = 0.05$.

2.4 Results

2.4.1 Soil health indicators

There were significant differences in POXC ($p = 0.01$) and ACE protein ($p < 0.001$) between the ROWs and the off-ROW, whereas the TSR effects on 24-h $\text{CO}_2\text{-C}$ were not significant (Table 2.1). POXC on the 6-yr ROW (685 mg kg^{-1}) was 24% lower than on the 12-yr ROW (902 mg kg^{-1}) and 10% lower than on the off-ROW (761 mg kg^{-1}). Similarly, POXC on the 9-yr ROW (745 mg kg^{-1}) was 17% lower than on the 12-yr ROW (902 mg kg^{-1}) and 2% lower than on the off-ROW (761 mg kg^{-1}). ACE protein on the 6-yr ROW was 25% lower than on the 12-yr ROW and 21% lower than on the off-ROW (Table 2.1). ACE protein on the 9- and 12-yr ROWs were not significantly different from that on the off-ROW. There was a general increase in POXC, ACE protein, and 24-h $\text{CO}_2\text{-C}$ with increasing TSR, although the increase in soil respiration was not significant (Table 2.1). ROW age (TSR) had a significant effect on SOC ($p = 0.02$) but had no effect on CEC, EC, and pH (Table 2.1). Soil organic C on the ROWs was 32% (6-yr ROW) and 35% (9-yr ROW) lower than that on the off-ROWS. TSR treatments effects were not significant for $\text{NO}_3\text{-N}$, available P, available K, $\text{SO}_4\text{-S}$, and Ca (data not presented).

Table 2.1 Effects of ROW age on selected soil health indicators and soil chemical properties.

	POXC	ACE protein	CO ₂ -C	SOC	CEC	EC	pH
	mg kg ⁻¹ soil	mg kg ⁻¹ day ⁻¹	g kg ⁻¹	cmol _c kg ⁻¹	mS cm ⁻¹		
TSR†							
6 yr	685 b‡	9.3 b	140 a	26.7 b	13.1 a	0.42 a	6.9 a
9 yr	745 b	10.2 ab	170 a	25.6 b	12.8 a	0.35 a	6.9 a
12 yr	902 a	12.4 a	190 a	36.4 a	14.6 a	0.39 a	7.1 a
Off-ROW	761 a	11.8 a	136 a	39.2 a	14.3 a	0.33 a	6.9 a
<i>P-value</i>	0.01	0.0004	0.06	0.02	0.11	0.08	0.09

†TSR, time since reclamation (ROW age); POXC, permanganate-oxidizable carbon; ACE protein, autoclaved citrate-extractable protein; CO₂-C, soil heterotrophic respiration; SOC, soil organic carbon; CEC, cation exchange capacity; EC, electrical conductivity

‡Means in a column followed by the same lower-case letter are not significantly different at $p < 0.05$ according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

2.4.2 Crop yield

Across sites, sampling years, and test crops, both relative grain yield (RGY) and relative biomass yield (RBY) were significantly ($p = 0.001$) lower on the 6-yr ROW than on the older (9- and 12-yr) ROWs, whereas the relative yields on the 9- and 12-yr ROWs were not significantly different (Table 2.2). At 12 yr post-reclamation, RGY was still 42.4% lower and RBY 36% lower on the ROW than on the off-ROW. Nonetheless, both yield components showed an increasing trend over the 6-yr (i.e., 6 to 12 yr TSR) chronosequence of ROW recovery.

Table 2.2 Effects of time since reclamation (TSR) on relative grain yield (RGY) and total biomass yield (RBY) on natural gas pipeline ROWs relative to the yield on off-ROW transects.

TSR [†]	RGY [‡]	RBY
6 yr	34.2 b§	42.6 b
9 yr	58.0 a	58.9 a
12 yr	57.6 a	64.0 a
<i>P-value</i>	0.001	0.001

[†]TSR, time since reclamation; RGY, relative grain yield; RBY, relative total biomass yield

[‡]RGY and RBY values are expressed as a percent (%) of yield on the off-ROW

§Means in a column followed by the same lower-case letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

2.4.3 Crop nutrient uptake and grain protein content

Crop N uptake increased significantly on the 9-yr ROW relative to the 6-yr ROW for oats, but there was no significant temporal change in N uptake for all the other crops (Table 2.3). For wheat, N uptake was 71% and 61% lower in the 6- and 12-yr ROWs, respectively, than in the off-ROW. For oats, N uptake was 34% lower on the 6-yr ROW than on the 9-yr ROW (by 34%) and

the off-ROWS (by 46%). Barley N uptake was 35% lower on the 6-yr ROW than on the off-ROW. For both oats and barley, N uptake did not differ significantly between the 9-yr ROW and the off-ROW. Nitrogen uptake by peas showed no significant response to TSR. Wheat P uptake was greater on the off-ROW than on the 6-yr ROW (by 247%) and the 12-yr ROW (by 206%) (Table 2.3). For oats, P uptake on the 6-yr ROW was 39% lower than on the off-ROW, whereas it improved to off-ROW levels 9 yr after reclamation. Pea P uptake on the 6-yr ROW was 48% lower than on the off-ROW. Within 9 yr after reclamation, pea P uptake on the ROWs was similar to that on the off-ROW. The TSR effect was not significant for barley P uptake. There was a significant TSR effect on grain protein content for barley and canola only (Table 2.3). Protein content in barley was 16% lower on the off-ROW and 20% lower on the 12-yr ROW than on the 6-yr ROW, but there was no significant difference between the 9-yr and 12-yr ROWs vs. the off-ROW or between any of the other ROW pairs. Canola grain protein content was significantly greater for the 12-yr ROW than the other three treatments, but none of the other treatment pairs were significantly different.

Table 2.3 Nitrogen and phosphorus accumulation in grain and straw and grain protein content as affected by pipeline ROW age.

Crop	TSR [†]	Total N uptake	Total P uptake	Grain protein
		kg ha ⁻¹		%
Wheat	6 yr	11.0 b‡	1.5 b	11.1 a
	9 yr	-§	-	-
	12 yr	14.5 b*	1.7 b	11.0 a
	Off-ROW	37.3 a	5.2 a	12.0 a
	<i>P-value</i>	0.001	0.001	0.1
Oats	6 yr	30.2 b	3.2 b	14.6 a
	9 yr	46.1 a	4.8 a	14.9 a
	12 yr	-	-	-
	Off-ROW	55.5 a	5.2 a	14.9 a
	<i>P-value</i>	0.002	0.01	0.7
Barley	6 yr	25.9 b	3.5 a	17.5 a
	9 yr	33.1 ab	4.6 a	15.7 ab
	12 yr	29.2 ab	5.3 a	14.0 b
	Off-ROW	40.0 a	5.7 a	14.7 b
	<i>P-value</i>	0.04	0.05	0.002
Peas	6 yr	73.8 a	5.3 b	20.0 a
	9 yr	109 a	9.0 ab	19.2 a
	12 yr	68.1 a	5.6 ab	19.7 a
	Off-ROW	140 a	10.2 a	19.8 a
	<i>P-value</i>	0.1	0.02	0.6
Canola	6 yr	56.3 ab	4.08 b	21.2 b
	9 yr	36 b	5.51 b	20.2 b
	12 yr	62.6 a	8.34 ab	23.8 a
	Off-ROW	57.5 ab	9.12 a	19.6 b
	<i>P-value</i>	0.03	0.03	0.001

[†]TSR, time since reclamation

‡Means in a column for each crop followed by the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

§The 9 yr ROW was not seeded with wheat, while the 12 yr ROW was not seeded with oats during the sampling years

*N and P uptake for 12-yr was determined using grain concentration only

2.5 Discussion

2.5.1 Soil health indicators

Overall, our results showed a general improvement in POXC and ACE protein with increasing TSR. These improvements are desirable on cropland as these soil health indicators positively correlate with soil productivity and the provision of ecosystem services (Doran and Zeiss, 2000). Despite the relative sensitivity of these soil health indicators to changes in land management (Culman et al., 2012; Hurisso et al., 2018; Weil et al., 2003), they have not been widely included in studies that evaluate the recovery of reclaimed ROWs on cropland. Of the few studies that included soil health assessment, most reported lower soil health indicator values on reclaimed ROWs than on the off-ROW. For example, Schindelbeck and van Es (2012) reported lower POXC and potentially mineralized nitrogen (PMN) on reclaimed ROWs than on the off-ROW on agricultural, wetland, and fallow landscapes. Unlike ACE protein (measured in our study) which reflects biologically available N, PMN encompasses different soil N pools (Schindelbeck et al., 2016; Hurisso and Culman, 2021). However, the two N indicators are highly correlated and both respond to changes in soil management (Cappellazzi & Morgan, 2021; Dungan et al., 2022).

The lower ACE protein values on the ROWs than on the off-ROWs observed in our study suggest that the reclaimed ROWs had smaller soil organic N pools than the off-ROWs, hence, lower long-term capacity to supply N to support plant life. Similar to ACE protein, POXC is a soil health indicator which is sensitive to changes in land use (Culman et al., 2012). While POXC can provide some insights into changes in labile C pools as a result of soil management, particularly those that deplete or build SOM (Culman et al., 2012), a recent study (Woodings and Margenot, 2023), showed that POXC does not represent labile C pools only since MnO_4^- can also be reduced by lignin a recalcitrant C pool. Lower POXC on the 6- and 9-yr ROWs than on the off-ROW could

be attributed to lower SOC observed on these ROWs relative to the off-ROW. Schindelbeck and van Es (2012) attributed the lower POXC and PMN on the ROWs to the mixing of biologically active topsoil with the nutrient poor subsoil. However, in this study, particle size analysis showed minimal horizon mixing on the ROWs. As a result, the lower POXC and ACE protein observed on the 6- and 9-yr ROWs in our study could not be attributable to horizon admixtures but to lower SOC observed on the ROWs relative to the off-ROW. In other studies POXC was also shown to positively correlate with TOC (e.g., Culman et al., 2012; Pulleman et al., 2021).

Our results indicate at least a gradual recovery of the three soil health indicators on the ROWs with increasing TSR, although the improvement in soil respiration was not significant. Soil respiration seemed to have recovered faster (within the first 6 yr post-reclamation) than POXC (12 yr) and ACE protein (9 yr). This could be due in part to the ameliorative impacts of agronomic practices on the ROWs, such as crop rotation, retention of crop residues, and minimum soil disturbance, which are known to help build the soil properties and, therefore, enhance recovery from a disturbance (Bagnall et al., 2023; Liptzin et al., 2023). These practices can slowly build soil C pools, which improve microbial communities and functioning, nutrient cycling, and overall soil functioning (Stetson et al., 2018; Williams et al., 2020; Van Eerd et al., 2021). Land management practices that increase organic C content and inputs into the soil increase microbial activity, thus soil respiration (Bastida et al., 2006; Bini et al., 2013). Bagnall et al. (2023) noted a significant increase in SOC and ACE protein under reduced tillage, cover cropping, and residue retention in a study conducted on 124 agricultural research sites across North America. Since POXC and ACE protein are also correlated with soil organic C (Weil et al., 2003; Liptzin et al., 2023), SOM additions from crop residues over time could also have resulted in the improvement in these indicators with increasing TSR. POXC and ACE protein showed a clear distinction between

ROWS of different TSR and the off-ROW. These findings indicate that these soil health indicators could be useful tools for assessing the recovery of productivity on ROWs on agricultural fields.

Soil organic C was lower on the 6- and 9-yr ROWs than on the 12-yr ROWs and the off-ROW. Our results corroborate those from other studies, which showed lower SOC on reclaimed ROWs than on the off-ROWS. For example, Soon et al. (2000a) showed that pipeline construction reduced SOC in the 0-10 cm layer but did not impact SOC in the 10-20 cm layer on ROWs on cropland in Alberta, Canada. Similarly, Brehm and Culman (2023) observed an average decrease of 6.5% in SOC on ROWs on cropland in Iowa, USA. Lower SOC on the ROWs compared to the off-ROWS could be due to reduced crop residue additions to the soil since crop biomass on reclaimed ROWs is typically lower than on the off-ROWS (Shi et al., 2015; Ebrahimi et al., 2022). Also, because of low productivity, reclaimed ROWs are usually not seeded during the first few years following reclamation, further reducing organic matter additions. However, the SOC concentration on the 12-yr ROWs was similar to that on the off-ROW, indicating an improvement in SOC as TSR increased. This could be attributed to accumulation of organic matter with increasing TSR since farmers at our study sites retain crop residues on their field after crop harvest. The addition of organic amendments either during reclamation or as a land management strategy post-reclamation has been shown to hasten the recovery of disturbed land by increasing SOC concentrations (Larney and Angers, 2012).

Variable results have been reported on the impacts of pipeline construction on plant available nutrients, with some studies showing a decrease (e.g., Shi et al., 2015; Brehm and Culman, 2023) and others showing no significant impacts of pipeline construction (e.g., Soon et al., 2000b; Gasch et al., 2016). It is noteworthy that, unlike non-cropland ecosystems, for instance, grassland, where inorganic fertilizers are often applied to stimulate plant growth during

revegetation (Desserud et al., 2010), inorganic fertilizers are applied yearly on cropland to support the desired crop yield goals. The continual application of inorganic fertilizers is expected to elevate plant available nutrients on the ROWs on cropland, thus confounding nutrient availability results. As such, plant-available nutrients on cropland might not be a reliable measure of the recovery of soil productivity after reclamation of ROWs.

Some studies showed higher soil pH and EC on the ROWs than on the off-ROWs on cropland (e.g., Soon et al., 2015; Shi et al., 2015; Whitson, 2023). Increased soil pH on the ROWs is usually evidence of soil horizon mixing during excavation, soil piling, and trench backfilling, particularly in soil profiles with calcareous subsoils (Soon et al., 2000b; Coiffait-Gombault et al., 2012). As a result, changes in soil pH usually coincide with changes in soil EC (De Jong and Button, 1973). Our results showed no significant TSR effect on pH and EC, suggesting minimal horizon mixing, which was consistent with the measured particle size distribution. In addition, unlike in the previous studies where soil properties were measured in soil samples collected from the trench area, where the magnitude of impact is greatest, our results represent the entire ROW width (all ROW zones), which could have diluted the measured impacts in our study. The extent of the adverse impacts of pipeline construction on soil properties varies with the ROW zone. It is expected that the impacts of pipeline construction on soil pH and EC would be greater in the trench area where soil excavation occurs and where horizon mixing is more likely than in the other zones of the ROW. In another study conducted in the agricultural regions of west China, the adverse effects of pipeline construction were most severe in the trench, followed by the piling and working areas and became less severe as distance from the trench area increased from 20 to 50 m (Shi et al., 2014).

2.5.2 Crop yield and nutrient content

Our results showed that crop biomass and grain yields were significantly lower on reclaimed ROWs than on the off-ROW even after 12 yr post reclamation. These results corroborate those of previous studies, which showed the detrimental impacts of pipeline construction on crop productivity (e.g., Culley and Dow 1988; Shi et al., 2015; Brehm and Culman, 2023). Lower crop yields on reclaimed ROWs relative to off-ROWs have been attributed to several factors, including increased bulk density and soil penetration resistance (Tekeste et al., 2019; Ebrahimi et al., 2022), topsoil dilution due to soil horizon mixing (Soon et al., 2000a; Shi et al., 2015), and reduced plant-available nutrients (Soon et al., 2000a; Brehm and Culman, 2023). The dilution of the relatively fertile topsoil by the subsoil reduces the concentration of plant available nutrients in the soil (Desserud et al., 2010; Shi et al., 2015). In addition, soil dilution can alter soil pH (Soon et al., 2000a), which can influence the availability of nutrients such as P for crop uptake. Unlike in the previous studies, particle size distribution, soil pH, and EC measurements in our study suggested that soil horizon mixing was minimal, hence could not have influenced the yields on the ROWs in our study. However, results from a companion study presented in Chapter 3 showed that soil penetration resistance, macroporosity, and saturated and near-saturated hydraulic conductivities were significantly lower on ROWs younger than 6.5 yr relative to the off-ROW which could have caused the yield losses on the ROWs observed in this study.

Although the uptake of N and P was lower on the 6-yr ROWs than on the off-ROWs, the grain concentrations of N and P for all crops and treatments did not indicate nutrient deficiency (Table S2.2). For example, the concentrations of P in wheat, oats, and barley were 2.37-4.04 g kg⁻¹, which are within the sufficiency range (1.3 to 3.9 g kg⁻¹ dry matter) for grain P concentration in cereals (Hoppe et al., 1999; Rashid et al., 2005). Similarly, grain N concentrations were 17.9-26.1

g kg⁻¹ and fell within the sufficiency range for instance 19 to 28 g kg⁻¹ dry matter for wheat (Nakano et al., 2010). Although there were significant differences among treatments for grain protein content for some crops, protein content was also within the desired ranges for all crops and all treatments, suggesting minimal impacts of pipeline construction on crop quality. For instance, protein content was within the 10-13% range recommended for baking wheat (Godfrey et al., 2010; Sharma et al., 2020). However, protein content was above the desired 9-12% range for malting barley (Rani and Bhardwaj, 2021), which could limit the enzymatic breakdown of starch, leading to poor malt extracts (Rani and Bhardwaj, 2021). On the other hand, low protein content impairs brewing due to poor amino acid nutrition for yeast (Rani and Bhardwaj, 2021). As such, high-protein barley fetches a lower price on the grain market (Sharma et al., 2020). Overall, our results did not show any negative impacts of pipeline construction on N and P uptake and grain protein content. These findings suggest that the crop yield losses observed on the ROWs relative to off-ROWs were unlikely due to nutrient deficiencies but may have been caused by restricted root growth due to high penetration resistance, water deficits in the root zone resulting from reduced soil water transport (Muitire et al., 2024) which can lead to low available soil water, hence, low crop water uptake on the ROWs.

Crop grain and biomass yields were still lower on the ROWs than on the off-ROWs 12 yr after ROW reclamation, suggesting that the impacts of pipeline construction on crop productivity are long-term. Nonetheless, our results showed a slow but progressive recovery of crop productivity on reclaimed ROWs with increasing TSR. These results corroborate other studies, which showed improvements in crop productivity on reclaimed ROWs with increasing TSR (e.g., Soon et al., 2000a; Shi et al., 2015; Brehm and Culman, 2023). These previous studies generally show that the impacts of pipeline construction on crop yields are long-term. For example, Brehm

and Culman (2023) observed that the impacts of pipeline construction on crop yield persisted for 5 yr after reclamation of underground natural gas pipeline ROWs in Ohio, USA. Our study fills a knowledge gap by including older ROWs than those evaluated in most of the previous chronosequence studies examining ROWs on cropland (e.g., 3 yr (Soon et al., 2000a), 5 yr (Brehm and Culman, 2023), and 8 yr (Shi et al., 2015)).

The improvement in crop productivity on reclaimed ROWs with increasing TSR has been attributed to the influence and interaction of natural soil recovery processes and good land management practices (e.g., Soon et al., 2000b; Gasch et al., 2014; Shi et al., 2015). Land management practices, such as minimum tillage, crop rotation, and retention of crop residues are practiced at the three study sites. These practices build SOC, increase labile C and N fractions, and encourage soil biodiversity which aid soil recovery (Nunes et al., 2020). These are the typical agronomic practices at our study sites and may explain the observed improvement in crop productivity with increasing TSR. Minimum tillage and the retention of crop residues build SOC content, which is crucial for improving soil structure and aggregation, and improve soil microbial abundance and functional diversity, hence nutrient cycling (Shi et al., 2015; Stetson et al., 2018; Van Eerd et al., 2021). Overall, these practices improve soil health (Nunes et al., 2020; Liptzin et al., 2022), which could translate to improved crop productivity on the ROWs, as observed in our study.

Reduced crop yields on reclaimed ROWs 12 yr post reclamation in our study indicate that the impacts of pipeline construction on crop productivity are long-lasting. These long-lasting impacts likely result from the presence and operation of the pipeline. One such impact is elevated soil temperature on the ROWs relative to the off-ROWs due to the heat generated by the flow of pressurized natural gas in the pipelines (Naeth et al., 1993; Halmová et al., 2017). Elevated soil

temperature alters soil moisture regimes, usually by increasing soil moisture loss through evaporation (Naeth et al., 1993; Halmová et al., 2017). Thus, yield losses due to lower available soil moisture content on the ROWs relative to the off-ROWs are expected to be greater during drought or in semi-arid regions such as northeastern British Columbia, where our study was located. Although temperature was not monitored in our study, it is conceivable that it could be contributing to the long-term impacts on crop yields observed in our study.

2.6 Conclusion

Results from this study showed that the construction of underground pipelines on cropland has detrimental impacts on crop yields and some soil attributes. Crop yields on reclaimed ROWs were at their lowest 6 yr after reclamation, with a significant improvement in crop yield as time since reclamation increased from 6 to 9 yr. However, 12 yr following reclamation, crop yield on the ROWs was still lower relative to yield on the off-ROW, clearly indicating that although productivity on the ROW would recover with time, the detrimental impacts of pipeline construction on crop yield are long-term. Soil chemical attributes seemed to have been minimally impacted by pipeline construction or could have fully recovered to off-ROW levels within 6 yr after reclamation. However, the status of soil chemical attributes, particularly plant available nutrients, could also have been confounded by fertilizer application on the ROWs. As a result, plant available nutrients might not be a reliable indicator of ROW recovery on cropland, unlike on other ecosystems, for instance, grassland, and forests where inorganic fertilizers are not continuously applied to aid vegetation growth. In this study, POXC and ACE protein concentrations were lower on the younger ROWs and improved with increasing TSR, closely matching those on the off-ROW 12 yr after ROW reclamation. Our results indicate that underground pipeline construction greatly reduces the capacity of cropland to support plant life,

although this capacity would improve with increasing time post-reclamation. The findings also demonstrate the potential of these soil health indicators in assessing the recovery of reclaimed underground pipeline ROWs on cropland. The impacts of pipeline construction seemed to extent for a longer period for crop yield than for soil chemical properties suggesting the influence of other underlying long-term impacts of pipeline construction. Such factors could include elevated soil temperature and concomitant low moisture due to the physical presence of the pipeline and its contents in the soil environment. Future studies should examine such effects and devise strategies to minimize heat transfer from the pipelines to the surrounding soil ecosystem.

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3. TEMPORAL CHANGES IN SOIL PHYSICAL PROPERTIES FOLLOWING RECLAMATION OF UNDERGROUND NATURAL GAS PIPELINE RIGHTS-OF-WAY ON CROPLAND

3.1 Abstract

Underground oil and natural gas pipeline rights-of-way (ROWs) sometimes traverse croplands, leaving behind tracts of disturbed land characterized by poor productivity even after reclamation. This study investigated temporal post-reclamation changes in soil penetration resistance (PR), field saturated hydraulic conductivity (K_{fs}), near-saturated hydraulic conductivity (K_{ψ}), soil pore indices, and wet aggregate stability (WAS) on natural gas pipeline ROWs on cropland. These attributes were measured in two fields in late summer of 2021 and 2022. Across the two fields, three ROWs that had been reclaimed four to thirteen years prior (i.e., time since reclamation (TSR) of 4 to 13 yr) were assessed along with adjacent undisturbed (off-ROW) transects located 50 m from the ROWs. Measurement locations on each ROW and on off-ROW were 25 m apart. Samples for WAS were collected from the 0-15 cm layer. The results showed a significant ($p < 0.05$) TSR by soil depth interaction for PR. Treatments were not significantly different in the 0- to 20-cm and 40- to 60-cm layers, but in the 20- to 40-cm depth interval, PR was 57.7-64.2% greater on the 4.5-yr ROW than on off-ROW. Saturated hydraulic conductivity was three to four times lower on the 4.5- and 6.5-yr ROWs than on the off-ROW. Near-saturated hydraulic conductivity on the 4.5-yr ROW was 61.6% lower than that on the off-ROW, while no significant differences were detected between the 6.5-yr and 12.5-yr ROWs and the off-ROW. Effective water conductive macroporosity was 72.5% lower on the 4.5-yr ROW than on the off-ROW. All measured physical properties improved with increasing TSR. These results indicate a

significant but slow recovery in soil physical attributes with increasing TSR of ROWs on cropland. This has implications on the productivity of these ROWs as crop yields, which depend on these soil physical properties, are also expected to recover slowly.

3.2 Introduction

Despite the increasing adoption of renewable energy sources, the global demand for oil and natural gas products is not expected to abate soon (Gong, 2020). In some regions, the demand for oil and natural gas products is increasing, putting pressure on producing regions (e.g. Canada) to expand exploration, development, and production despite concerted efforts to cut down greenhouse gas emissions. Canada is among the major global producers of crude oil and natural gas, ranking fourth and fifth, respectively, in 2021 production volumes (Natural Resources Canada, 2022). To meet the increasing global demand for oil and natural gas products, exploration and production are increasing along with the construction and expansion of associated infrastructure such as pipelines. Pipelines are preferred for short and long distance transportation of crude oil and natural gas due to their efficiency, safety, and cost effectiveness (Desserud et al., 2010; Olson & Doherty, 2012). Where possible, the installation of crude oil and natural gas pipelines avoids agricultural land, wetlands, and any potentially unstable land due to their impacts on soil and vegetation ecosystems (Naeth et al., 1987; Culley and Dow, 1988; Shi et al., 2014). However, due to logistical considerations, underground pipeline rights-of-way (ROWs) sometimes traverse cropland, inevitably leaving behind tracts of disturbed land characterized by poor productivity (Shi et al., 2015). A pipeline ROW is the total area that is impacted by pipeline construction. It typically consists of the work area (~20 m wide) where construction vehicles move and temporary storage of materials occur, the trench area (~5 m wide) where excavation and pipeline laying are done, and the piling area (~20 m wide) where excavated soil is salvaged before

backfilling. The width of ROWs can extend to 50 m. However, the ROW width varies with factors such as the size of the pipeline, the constructor, and equipment used. To minimize the pipeline footprint on the environment, constructors usually overlap the ROWs.

Pipeline construction activities, particularly movement of heavy construction vehicles and mixing of soil horizons, can adversely impact soil physical properties, resulting in reduced soil functionality and productivity (Tekeste et al., 2020). Heavy vehicles with an axle load exceeding 10 t per axle can result in extensive soil compaction, with detrimental impacts on crop productivity (Schjønning & Rasmussen, 1994). Soil compaction reduces both the size and continuity of meso- and macroporosity and alters their shape as well (Weil & Brady, 2017). Consequently, steady-state infiltration rates, hydraulic conductivity, and water retention are greatly reduced on pipeline ROWs (Soon et al., 2000), while bulk density (BD), soil strength, and penetration resistance (PR) tend to increase (Tekeste et al., 2020).

In a cropland study in Iowa, USA, Tekeste et al. (2020) reported a significantly greater BD (mean = 1.52 Mg m^{-3}) on a pipeline ROW relative to undisturbed sites on either side of the ROW ($1.29\text{-}1.44 \text{ Mg m}^{-3}$) in the 0-50 cm soil layer. The same study also showed greater PR on the ROW compared to undisturbed parts of the field 2 yr after subsoiling the ROW. These impacts were attributed to vehicular traffic on the ROW during pipeline construction. Changes in soil porosity due to vehicle-induced compaction impacts soil water percolation in the soil profile. Ramezani et al. (2017) reported a 17.3% decrease in saturated hydraulic conductivity compared to the control in a single tractor pass treatment and a 71% decrease for an 8-pass treatment. Reduced water infiltration in a compacted soil profile enhances surface water runoff, which increases the risk of soil and nutrient loss via erosion while reducing the replenishment of groundwater storage (Horton et al., 1994).

In crop production systems, soil compaction is highly undesirable. It impedes crop root growth and development due to increased PR, poor soil aeration, slow water and nutrient movement, mechanical impedance, and buildup of toxic gases and root exudates (Weil and Brady, 2017). Inhibited crop rooting impacts water and nutrient uptake and crop anchorage, thus adversely impacting crop productivity (Weil and Brady, 2017; Tekeste et al., 2020). Negative linear correlation of crop root growth and soil PR have been reported for oats (Ehlers et al., 1983), corn (He et al., 2017), and soybean (Moraes et al., 2020) with yield reductions of 9-50% reported for corn and soybean on pipeline ROWs compared to adjacent undisturbed parts of the field (Tekeste et al., 2020). Varying maximum soil PR values for uninhibited crop root development have been reported (e.g., Barbosa et al., 2018; Moraes et al., 2020), depending on soil properties (e.g., soil type, moisture content, and organic matter content) and crop species (Bartzen et al., 2019; Moraes et al., 2020).

Mechanical and agronomic techniques to ameliorate soil compaction on croplands have been proposed, with subsoiling to a depth of 45 cm reported as the most effective practice in breaking up dense soil layers in the crop root zone (Spoor, 2006). As a result, soil compaction on the ROW could be a bigger concern in agricultural systems where zero or minimum tillage is practiced than in systems with periodic soil disturbance through tillage. Soil compaction takes decades to naturally ameliorate through plant root activity and other biological processes (Batey, 2009). In pipeline construction, approaches to construct underground pipelines in a more responsible way to minimize negative impacts on soil attributes are being implemented. These include constructing pipelines in winter when the soil is frozen (Neilsen et al., 1990), salvaging and backfilling excavated soil according to horizons (double-lift technique) (Desserud et al., 2010), and use of relatively lighter construction vehicles (Tekeste et al., 2019).

Although the impacts of pipeline construction on soil physical properties have been reported (e.g., Soon et al., 2000; Tekeste et al., 2019; Brehm and Culman, 2023), there is a dearth of information on the recovery of these properties with increasing time since reclamation (TSR) of natural gas pipeline ROWs on cropland. Determining the impacts of pipeline construction activities on soil physical properties and the changes that occur to these attributes with increasing TSR will allow stakeholders to make informed decisions on appropriate land management techniques to quickly ameliorate the impacts and could aid in the development of landowner compensation plans. Landowners whose properties are impacted by pipeline construction activities are usually compensated by industry for loss in productivity on pipeline ROWs (Brehm and Culman, 2023). The compensation packages are mere agreements between the landowner and the pipeline company informed by land appraisals (Carr-Wilson, 2014). This can lead to conflicts between pipeline construction companies and landowners as some landowners may feel that they are not being adequately compensated (personal communication with farmers, 2021). Evaluating changes to soil properties will provide a scientifically-sound basis that will support accurate determination of loss in crop production.

The objectives of this study were to determine (i) the impacts of pipeline construction activities on soil physical properties (penetration resistance, saturated hydraulic conductivity, near-saturated hydraulic conductivity, soil pores, and wet aggregate stability) and (ii) the changes in these soil attributes with increasing TSR of the ROWs. We hypothesized that saturated hydraulic conductivity, near-saturated hydraulic conductivity, aggregate stability, and soil pore indices would be lower on reclaimed ROWs while penetration resistance would be higher on reclaimed ROWs than on adjacent undisturbed parts of the field and that the state of these soil attributes on reclaimed ROWs would improve with increasing TSR.

3.3 Materials and Methods

3.3.1 Site characteristics

The study was conducted in Farmington (55° 54' N; 120° 30' W), northeast British Columbia, Canada. The long-term (1981-2010) mean temperature for the area is 13 °C during the growing season (May to September) and -10.1 °C during winter (November to February). Mean annual precipitation is 445 mm (Environment and Climate Change Canada, 2024). The study site is within the Peace region, which is home to one third of the land in British Columbia's 4.7 million ha Agricultural Land Reserve (ALR, a zone where agriculture is recognized as the top priority land use and non-agricultural land uses are restricted) (B.C. Minister of Agriculture's Advisory Committee, 2018). The area has the greatest agricultural productivity in the province, including crop, fodder, and animal production. The most common crops are canola (*Brassica rapa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and field pea (*Pisum sativum* L.), which are normally grown in a legume-cereal-canola rotation. Conservation tillage is common in the area, and farmers participating in the study use zero-till drills with hoe-type openers for seeding. They also practice crop residue retention with harrowing to even out crop stubbles with periodic subsoiling. The ALR produces food for local consumption and export, accounting for 85% of British Columbia's farm receipts (B.C. Government News, 2018). Non-agricultural land uses such as roads, railways, and pipeline ROWs are inevitably impacting the potential for agricultural production in this area, increasing the need for reclamation and remediation. The Farmington area has level to near-level slopes and moderate to good surface drainage (B.C. Soils Information Finder Tool, 2018). Site 1 was at an elevation of 757.7 m with an average slope of 2.6% while Site 2 was at an elevation of 766.3 m with an average slope of 2.3% (Google Earth Pro, 2023). The

dominant soils at the study sites are Orthic Gray Luvisols (Typic Cryoboralf) with a silt loam texture and an acid to neutral pH (B.C. Soils Information Finder Tool, 2018).

3.3.2 Pipeline rights-of-way

Measurements and soil samples were taken from three underground pressurized natural gas pipeline ROWs of TSR 4-5 (hereinafter, 4.5), 6-7 (6.5), and 12-13 (12.5) yr and from adjacent undisturbed parts of each field (off-ROW) at two locations during August-September in 2021 and 2022. All three pipelines were installed at a depth of 1.2-2 m.

3.3.3 Sampling design

Four sampling and measurement locations (replicates) of area 4 m² each were marked on each pipeline ROW and off-ROW from where measurements and soil samples were taken. Penetration resistance, saturated hydraulic conductivity, and near-saturated hydraulic conductivity were measured in the field. Hydraulic conductivity measurements were taken on three locations while all four locations were used for penetration resistance measurement. The measurement locations were marked avoiding visible vehicle tracks to minimize confounding due to the effect of farm traffic. Sampling locations on each ROW and on off-ROW were 25 m apart. The off-ROW transects were 50 m from the ROWs. Soil samples were collected from four sampling locations using a Dutch auger for determination of wet aggregate stability and soil chemical properties.

3.3.4 Measurements

3.3.4.1 Soil characterization

Particle size distribution (PSD) was determined using the hydrometer method (Gee and Or, 2002). Soil organic matter (SOM) content was measured using the loss on ignition (360 °C) method (Combs & Nathan, 2015). Total carbon (TC) was determined using the dry combustion method (Nelson and Sommers, 1996) while inorganic carbon was estimated by pressure calcimetry after reacting the soil with 15% HCl (Loeppert and Suarez, 1996). Soil organic carbon (SOC) was calculated as the difference between total carbon and inorganic carbon (Nelson and Sommers, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:2 soil:water paste according to Peters et al. (2015) and Whitney (2015), respectively.

3.3.4.2 Penetration resistance

Penetration resistance (PR) was measured using a hand-held penetrometer (Eijkelkamp PV6.08, Netherlands) fitted with a 1 cm² and 60° top angle cone. The penetrometer tip and rod simulates root elongation down the soil profile (Bartzen et al., 2019). In this study, measurements were taken to a depth of 60 cm from four random spots in each of the four sampling areas, giving a total of 16 measurements per treatment. Measurements were taken by manually pushing the penetrometer tip and rod vertically into the soil maintaining a constant penetration speed.

3.3.4.3 Near-saturated hydraulic conductivity

Near-saturated hydraulic conductivity (K_{ψ}) was measured at two supply pressure potentials (-3.0 and -0.5 cm H₂O) using a mini disk infiltrometer (METER Group Inc., USA). The supply pressure potentials we used in this study allowed us to estimate changes to soil macroporosity due to pipeline installation and time since reclamation. The instrument was set up at locations that were

prepared by carefully removing all vegetation, litter, crop stubs, and stones that would interfere with achieving a level surface. Preparation of the spot was done with minimal soil surface disturbance. Six measurements were taken at 15-min intervals for the two supply pressure potentials in ascending Ψ (wetting) sequence while maintaining the infiltrometer position. The ascending Ψ sequence prevents hysteresis effects which are associated with the descending Ψ sequence (Zvomuya et al., 2009). Measurements were taken at three sampling locations (replications) per treatment.

Infiltration measurements from the disk infiltrometer, the soil texture, and the respective supply pressure potentials were then used to compute K_Ψ according to the method described by Zhang (1997). The method utilizes the slope (C_1) obtained from fitting cumulative infiltration (I) versus the square root of time. Cumulative infiltration is determined using the two-term function (Eq. [3.1]) that describe the infiltration process under the disk infiltrometer at any infiltration time:

$$I = C_1 t + C_2 t^{1/2} \quad [3.1]$$

where C_1 (m s^{-1}) and C_2 ($\text{m s}^{-1/2}$) are curve fitting parameters related to hydraulic conductivity (C_1) and soil sorptivity (C_2) and t is time (s)

Near-saturated hydraulic conductivity is then computed using Eq. [3.2]:

$$K_\Psi = \frac{C_1}{A} \quad [3.2]$$

where C_1 is the slope of I versus $\sqrt{t}$ and A is a nondimensional coefficient relating the van Genuchten parameters for a given soil texture to the suction and radius of the infiltration disk (Carsel & Parrish, 1988). Values of A are computed from Eqs. [3.3] and [3.4]:

$$A = \frac{11.65 (n^{0.1}-1) \exp [2.92(n-1.9)\alpha h_0]}{(ar_0)^{0.91}} \quad n \geq 1.9 \quad [3.3]$$

$$A = \frac{11.65 (n^{0.1}-1) \exp [7.5(n-1.9)\alpha h_0]}{(ar_0)^{0.91}} \quad n < 1.9 \quad [3.4]$$

where n and α are the van Genuchten retention parameters for the soil, r_0 is the disk radius of the infiltrometer, and h_0 (≤ 0) is the suction at the disk surface (Zhang, 1997).

3.3.4.4 Saturated hydraulic conductivity

Saturated hydraulic conductivity (K_{fs}) measurements were taken using the SATURO infiltrometer (METER Group Inc., USA). On each of the three measuring locations (replicates) per treatment, a spot was randomly selected for measurement of K_{fs} . The SATURO uses the two-ponding head approach described by Reynolds and Elrick (1990) with modifications to correct for errors associated with the single and double-ring infiltrometer. The soil surface was prepared using the procedure described above in Section 3.3.4.3 for the tension infiltrometer measurement. A 5-cm insertion ring was gently pushed into the soil, and the instrument was then set up for measurements. The SATURO was set to run for three cycles on each location using the following settings: 25 min soak time, 5 cm pressure head, 10 cm pressure head, and 15 min hold time at each pressure. These settings resulted in a total run time of 115 min for each measurement. One measurement was taken per sampling location. Using the two-ponding head approach, K_{fs} was computed using Eq. [3.5]:

$$K_{fs} = \frac{i}{F} \quad [3.5]$$

where i (cm/s) is the steady (final) infiltration rate (volume divided by area) and F is a function that corrects for sorptivity and geometrical effects (Nimmo et al., 2009).

3.3.4.5 Aggregate stability

Soil samples for aggregate stability measurement were collected using a spade from the 0–15 cm layer at each of the four sampling locations. Aggregate stability was determined using two water stable aggregate indices: mean weight diameter (MWD) and percent stable aggregates

(PSA). PSA was measured using a wet sieving apparatus (Royal Eijkelkamp, Netherlands) following the procedure described by the equipment manufacturer while the Yoder device was used to determine MWD based on Eq. [3.6]:

$$MWD = \sum_{i=1}^n XW_i \quad [3.6]$$

where X is the mean diameter of the aggregates remaining on the respective sieves used, W_i is the ratio of the weight of the aggregates remaining on the sieve to the total weight of the sample, and n is the number of sieves used for aggregate separation (Kemper and Rosenau, 1986).

3.3.4.6 Pore indices

Data collected using the minidisk infiltrometer were used to calculate soil pore indices at the two water supply pressure potentials (-3.0 and -0.5 cm). The maximum equivalent pore radii (r) which remained water conductive at either of the two supply pressure potentials was calculated using the classical capillary rise theory (Watson and Luxmore, 1986):

$$r = -\frac{2\sigma\cos\alpha}{\rho g\Psi}, \Psi < 0 \quad [3.7]$$

where α is the contact angle between the water and the pore wall (assumed 0), σ is the surface tension of water at 20 °C ($72.8 \times 10^{-3} \text{ kg s}^{-2}$), ρ is the density of water (998 kg m^{-3}), g is the acceleration due to gravity (9.81 m s^{-2}), and Ψ is the supply pressure potential in cm.

We determined the representative mean pore-radius for the two supply pressure potentials according to the theory outlined by Ankeny (1992) and Moret and Arr  e (2007):

$$\lambda_{\Delta\Psi} = \frac{\sigma(K_{\Psi_2} - K_{\Psi_1})}{\rho g(\Phi_{\Psi_2} - \Phi_{\Psi_1})} \quad [3.8]$$

where K_{Ψ} is the hydraulic conductivity (cm s^{-1}) for the two consecutive supply pressure potentials, and $\Phi_{\Psi_1} - \Phi_{\Psi_2}$ is the difference in matrix flux potential between the two supply pressure potentials, computed as:

$$\Phi_{\psi_1} - \Phi_{\psi_2} = \frac{(\psi_2 - \psi_1)(K_{\psi_2} - K_{\psi_1})}{\ln(K_{\psi_2}/K_{\psi_1})} \quad [3.9]$$

The maximum number of effective water-conductive pores per unit area ($N_{\Delta\psi}$, m⁻²) was calculated according to Eq. [3.10] (Moret and Arr  , 2007):

$$N_{\Delta\psi} = \frac{8\eta(K_{\psi_2} - K_{\psi_1})}{\pi\rho g(\lambda_{\Delta\psi})^4} \quad [3.10]$$

where η is the dynamic viscosity of water (0.01002 g cm⁻¹ s⁻¹).

The effective water-conductive porosity ($\theta_{\Delta\psi}$, %) was calculated as:

$$\theta_{\Delta\psi} = N_{\Delta\psi}\pi(\lambda_{\Delta\psi})^2 \times 100 \quad [3.11]$$

3.3.5 Statistical analysis

Analysis of variance of all data was performed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS[®] OnDemand for Academics (SAS Institute Inc, 2023). Penetration resistance (PR) data were subjected to repeated measures analysis, with TSR and sampling depth interval modelled as fixed effects, while site and block nested within site were random effects. The repeated measures effect of depth was modelled using the Toeplitz (TOEP) covariance structure, which was selected, based on the Akaike Information Criterion (AIC), ahead of the other eligible covariance structures - compound symmetry (CS), first-order autoregressive (AR[1]), Toeplitz (TOEP), and their heterogenous covariance forms (CSH, ARH[1], and TOEPH). Data distributions were Gaussian for penetration resistance and MWD, beta for PSA, and gamma for saturated and near-saturated hydraulic conductivity. Treatment means were compared using the Tukey-multiple comparison procedure at $\alpha = 0.05$ for all variables except saturated hydraulic conductivity, which showed a low statistical power (0.62) and therefore had its effects assessed at $\alpha = 0.1$.

3.4 Results

3.4.1 Selected soil chemical properties

The soils on the ROWs contained 27-31% sand, 46-52% silt, and 21-27% clay in the 0-15 cm layer and 21-29% sand, 36-50% silt, and 35-41% clay in the 15-60 cm layer (Table 3.1). The average particle size fractions on the off-ROW transects were 29% sand, 47% silt, and 24% clay in the 0-15 cm layer and 25% sand, 44% silt, and 31% clay in the 15-60 cm layer (Table 3.1). Soil organic matter content in the 0-15 cm soil layer was significantly ($p = 0.004$) greater on the off-ROW transects than on the ROWs (Table 3.1). The 4.5-yr ROW had the lowest SOM concentration of all treatments. Treatments were not significantly different with respect to soil pH and EC.

Table 3.1 Selected soil physical and chemical properties on the rights-of-way and off-ROW locations.

TSR	PSF†						SOM	SOC	pH		EC	
	Sand	Silt	Clay	Sand	Silt	Clay						
	0-15 cm			15-60 cm								
	%											
							0-15 cm	0-15 cm	0-15 cm	15-60 cm	0-15 cm	15-60 cm
							g kg ⁻¹				dS m ⁻¹	
Off-ROW	29	47	48	25	44	31	59.4±2.6 ‡	32.7±1.6	7.0±0.1	7.0±0.05	0.3±0.08	0.3±0.08
4.5 yr	31	46	23	21	38	41	42.8±1.1	22.3±1.0	6.9±0.1	7.0±0.08	0.4±0.09	0.4±0.09
6.5 yr	29	46	25	29	36	35	49.3±1.3	28.2±0.7	7.1±0.1	7.0±0.08	0.2±0.09	0.2±0.09
12.5 yr	27	52	21	27	36	37	44.0±2.0	24.9±1.1	6.9±0.1	7.0±0.08	0.3±0.09	0.3±0.09

†PSF, particle size fractions; SOM, soil organic matter; SOC, soil organic carbon; pH, soil pH; EC, electrical conductivity

‡Treatment least square means for SOM, SOC, pH, and EC are ± standard error of the mean

3.4.2 Penetration resistance

There was a significant ($p = 0.02$) TSR $\times$ soil depth interaction for PR (Table 3.2). Penetration resistance in the 20- to 40-cm soil layer was significantly greater on the 4.5-yr ROW than on the off-ROW, but the TSR effect was not significant in the 0- to 20-cm and the 40- to 60-cm soil layers (Figure 3.1). Penetration resistance on the 4.5-yr ROW was 57.7% higher in the 20- to 30-cm soil layer and 64.2% higher in the 30- to 40-cm soil layer than on the off-ROW. The PR in the 6.5- and 12.5-yr ROWs were not significantly different from that in the off-ROW in all soil layers. It is noteworthy that the 4.5-year ROW had numerically greater PR than the other TSRs in all soil layers (Figure 3.1).

Table 3.2 Mean penetration resistance values on the rights-of-way as affected by time since reclamation and soil depth.

Treatment	Penetration resistance (MPa)
TSR [†] (yr)	
4.5	4.5 a [‡]
6.5	3.2 b
12.5	2.7 b
Off-ROW	3.4 b
Soil depth (cm)	
5	1.4 d
15	3.3 c
25	3.7 b
35	3.9 ab
45	4.1 ab
55	4.3 a
	—— <i>P-value</i> ——
TSR	0.01
Soil depth	0.0001
TSR $\times$ Soil depth	0.02

[†]TSR, time since reclamation in years

[‡]Means in a column followed by the same letter are not significantly different at $\alpha \leq 0.05$ according to Tukey's multiple comparison test

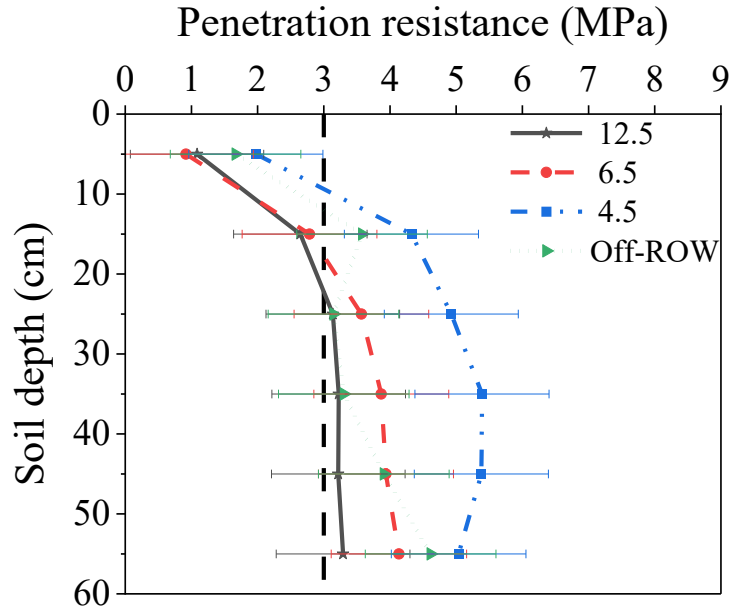


Figure 3.1 Changes in soil penetration resistance with soil depth as affected by time since reclamation. Error bars represent standard errors of the means.

3.4.3 Hydraulic conductivity

Near-saturated hydraulic conductivity (K_{Ψ}) was three times significantly ($p < 0.0001$) higher at the -0.5 cm than at the -3.0 cm supply pressure potential (Ψ) across all four treatments (Table 3.3). Averaged across the two pressure potentials, there was a significant ($p = 0.001$) TSR effect on K_{Ψ} . Mean K_{Ψ} on the 4.5-yr ROW was 61.6% lower than on the off-ROW, while no significant differences were detected between the 6.5- or 12.5-yr ROW and the off-ROW.

Time since reclamation had a significant ($p = 0.06$) effect on saturated hydraulic conductivity (K_{fs}) (Table 3.3). While K_{fs} did not differ significantly among ROWs, it was significantly greater on the off-ROW than on the 4.5- and 6.5-yr ROWs. The K_{fs} was four times lower on the 4.5- and three times lower on the 6.5-yr ROW than on off-ROW, while the 12.5-yr ROW was not significantly different from the off-ROW. Our results showed an increasing trend for K_{fs} with increasing TSR such that the K_{fs} values on the ROWs became more similar to the

values observed on off-ROW with increasing TSR (Table 3.3). Single degree of freedom contrast analysis revealed a significant difference between the mean K_{fs} of ROWs and the K_{fs} of off-ROW transects, suggesting the negative impact of underground pipeline construction on water flow down the soil profile under saturated conditions.

3.4.4 Soil macropore indices

Based on the classical capillary theory, the maximum equivalent pore radii (r) which remained water conductive at either of the two supply pressure potentials were 0.05 cm at the -3.0 cm supply pressure potential and 0.3 cm at the -0.5 cm supply pressure potential. Macropores are defined as soil pores with $r > 0.0375$ cm (Clothier and White, 1981) or pores that drain at $\Psi > -4.0$ cm (Moret and Arr  , 2007). Thus, pore size measurements in this study were limited to water-conductive macroporosity only.

Representative mean pore radius ($\lambda_{\Delta\Psi}$), which describes the average pore size participating in soil water transport (Moret and Arr  , 2007) and the number of macropores per m^2 ($N_{\Delta\Psi}$), which describes the number of effective water conductive macropores per area, did not differ significantly among treatments, although both indices were numerically lower on the 4.5-yr ROW (Table 3.3). Nonetheless, it is noteworthy that, with only three replicates for these measurements, the statistical power was very low (power = 0.19 for $\lambda_{\Delta\Psi}$ and 0.06 for $N_{\Delta\Psi}$). Retrospective power analyses showed that it would require 8 replicates for $\lambda_{\Delta\Psi}$ (standard deviation, SD = 0.02) and 15 replicates for $N_{\Delta\Psi}$ (SD = 1907) to attain the recommended minimum statistical power of 0.8 at $\alpha = 0.05$. As such, our ability to detect treatment effects was curtailed by the low statistical power for these soil attributes.

Effective water conductive macroporosity ($\theta_{\Delta\psi}$) accounted for a small proportion (<1%) of the total porosity of the soil on the treatments (Table 3.3). The $\theta_{\Delta\psi}$ was 72.5% lower on the 4.5-yr ROW (0.01%) than on the off-ROW (0.04%). As TSR increased, $\theta_{\Delta\psi}$ also increased such that by 6.5 yr after ROW reclamation $\theta_{\Delta\psi}$ had improved to a level which was statistically similar to that on the off-ROW, with no further increase as TSR increased to 12.5-yr (Table 3.3).

3.4.5 Aggregate stability

Although treatment effects were not significant, PSA on the 4.5-yr ROW was 31% less than that on the off-ROW (Table 3.3). Both PSA and MWD showed low statistical power (0.18 for PSA and 0.34 for MWD compared to a recommended minimum of 0.8) due to the low replication (three) employed, therefore, least square means for MWD were compared at $\alpha = 0.1$. Post-hoc sample analysis indicated that a minimum of six replicates would be needed for PSA to attain the requisite statistical power of 0.8 at $\alpha = 0.05$ and SD = 0.17.

Table 3.3 Effect of time since reclamation of natural gas pipeline rights-of-way on hydraulic conductivity, soil pores, and aggregate stability.

Parameter	$K_{\psi}^{\dagger}$	$\lambda_{\Delta\psi}$	$N_{\Delta\psi}$	$\theta_{\Delta\psi}$	K_{fs}	PSA	MWD
TSR, yr	cm h ⁻¹	cm	m ⁻²	%	cm h ⁻¹	%	mm
4.5	3.92 b $\ddagger$	0.011 a	564 a	0.01 b	5.29 b	51.5 a	0.64 b
6.5	11.7 a	0.03 a	2612 a	0.04 a	6.70 b	65.4 a	1.12 a
12.5	10.8 a	0.037 a	877 a	0.04 a	8.80 ab	76.1 a	0.82 ab
Off-ROW	10.2 a	0.049 a	835 a	0.04 a	19.57 a	82.2 a	0.88 ab
Suction (cm)							
-0.5	14.4 a	-	-	-	-	-	-
-3.0	4.92 b	-	-	-	-	-	-
	<i>P-value</i>						
TSR	0.001	0.22	0.73	0.04	0.06	0.22	0.06
Suction	<.0001	-	-	-	-	-	-
TSR $\times$ Suction	0.25	-	-	-	-	-	-

$\dagger K_{\psi}$, near-saturated hydraulic conductivity; $\lambda_{\Delta\psi}$, mean pore radius; $N_{\Delta\psi}$, number of macropores; $\theta_{\Delta\psi}$, effective water-conductive macroporosity; K_{fs} , field saturated hydraulic conductivity; PSA, percent stable aggregate; MWD, mean weight diameter; TSR, time since reclamation in years

$\ddagger$ Means in a column followed by the same letter are not significantly different at $\alpha \leq 0.05$ for K_{ψ} and $\theta_{\Delta\psi}$ and $\alpha \leq 0.1$ for K_{fs} and MWD according to Tukey's multiple comparison test

3.5 Discussion

3.5.1 Selected soil chemical properties

Results from this study indicated greater SOC (hence SOM) concentration on the off-ROW transects than on the ROWs, with the 4.5-yr ROW having the lowest SOC concentration. Similar to our results, Shi et al. (2015) reported higher SOM concentrations on off-ROW locations than on the ROWs on cropland. Similarly, Brehm and Culman (2023) did a quantitative analysis of 21 studies from across the world and reported an average decrease of 20.8% in SOC on the ROWs. Depleted SOM on the ROWs is typically attributed to admixtures between the SOM-rich topsoil and the relatively SOM-poor subsoil during topsoil scrapping, excavation, and soil piling (Naeth

et al., 1987; Shi et al., 2015) and increased carbon mineralization following soil excavation (Mason et al., 2011). The double-lift technique (carefully separating soil horizons during excavation, piling, and backfilling) minimizes the admixing of soil from different horizons (Desserud et al., 2010). However, admixtures are inevitable during underground pipeline construction (Desserud et al., 2010). Although admixing of topsoil and subsoil could be a contributing factor to lower SOC on the ROWs in this study, particle size analysis results suggest minimal admixing of soil horizons. As a result, lower SOC on the ROWs could be attributed mostly to lower additions of crop residues due to low crop yields on the ROWs and loss of topsoil during pipeline construction. Reduced crop yields on ROWs relative to the off-ROW transects have been reported in previous studies (e.g., Soon et al., 2000; Shi et al., 2015; Brehm and Culman 2023).

Elevated soil pH and EC on the ROWs often indicate admixing of soil horizons during pipeline construction and ROW reclamation, especially where the subsoil is calcareous and rich in carbonates (Soon et al., 2000; Coiffait-Gombault et al., 2012). In both the topsoil and the subsoil in our study, soil pH and EC on the ROWs were similar to those on the off-ROWs, suggesting minimal admixing of the two soil layers. Our findings are consistent with those of Duncan and DeJoia (2011), who did not observe any changes in soil pH and EC on reclaimed ROWs relative to the off-ROW. In contrast, some studies showed increased soil pH on reclaimed ROWs. For instance, in a meta-analysis involving 26 pipeline ROW studies, Birkhimer et al. (2023) reported an average pH increase of 12% on reclaimed underground pipeline ROWs.

The effect of TSR on the recovery of SOM and SOC on the ROWs was evident albeit minimal. This was not surprising considering that Naeth et al. (1987) estimated that it would take 50 yr to restore 50% of the SOM lost due to underground pipeline construction on a Solonchic soil under native grassland. Accumulation of SOM, however, is expected to be higher on cropland

than on native grassland due to higher plant biomass accumulation in a shorter period of time on cropland.

3.5.2 Penetration resistance

Penetration resistance increased with soil depth on all treatments. However, PR was greater on the 4.5-yr ROW than on the 6.5- and 12.5-yr ROWs and the off-ROW in all soil layers. These results suggest that the impacts of pipeline ROWs on PR are more pronounced within the first 4.5-yr following reclamation but slowly ameliorate with increasing TSR. These findings corroborate several other studies who demonstrated PR values as high as 60% on the ROW than on the off-ROW (e.g., Culley et al., 1982; Soon et al., 2000; Tekeste et al., 2019). The elevated PR on the ROWs observed in these studies was associated with extensive soil compaction due to heavy pipeline construction equipment traffic, particularly in the work and trench zones of the ROW. The impact of underground pipeline construction on PR varies with soil texture, with medium- to fine-textured soils more prone to increased PR from underground pipeline construction than course-textured soils (Culley et al., 1982). Similar to these studies, the elevated PR observed on the 4.5-yr ROW in our study could be attributed to the immediate impacts of pipeline construction activities on the soil, particularly vehicle-induced compaction, which may have been exacerbated by the silty-loam to clay textural class of the soils on the study sites.

Penetration resistance is a crucial soil attribute in crop production systems because it reflects the resistance faced by plant roots as they penetrate the soil during their growth and development. There is an inverse relationship between the rate of plant root growth and PR (Ehlers et al., 1983; Bengough et al., 2001). High PR exposes plant roots to high resistance, thereby limiting the ability of the roots to access plant available nutrients and water (Bengough et al., 2001). However, root growth is a function of several other factors, including soil moisture content,

soil porosity, soil texture, nutrient availability, soil pH, and crop species (Bengough et al., 2001; Barbosa et al., 2018; Moraes et al., 2020). High PR also hinders seed germination and seedling emergence (Bengough and Mullins, 1991), which can ultimately reduce soil productivity.

Penetrologger measurements are exaggerated by a factor of between 2 and 8 relative to the penetration resistance encountered by plant roots. This is because the penetrometer tip and rod encounter greater frictional resistance during penetration whereas crop roots tend to follow crevices and soil pores and wind around soil particles and impediments, which substantially reduce the resistance faced by roots (Bengough and Mullins, 1991; Bengough et al., 2001). Therefore, PR values serve as a guide for uninterrupted root growth and fall in the category of soil attributes for which “smaller is better”. Different critical PR values at which plant root growth is restricted have been identified (e.g., Whalley et al., 2007; Moraes et al., 2014). Greacen et al. (1969) reported a critical range of 0.8-5.0 MPa, depending on crop and soil conditions. When using the penetrometer, a PR of approximately 3.0 MPa is regarded as the upper limit for uninterrupted root growth (Royal Eijkelpkamp, 2021). Our study showed greater PR values ranging from 3.0 to 5.5 MPa in the 10- to 60- cm soil layer on the 4.5- and 6.5-yr ROWs, indicating potential restriction to root growth and development within 6.5 yr post-reclamation, which can potentially reduce crop productivity.

3.5.3 Hydraulic conductivity

Most studies that evaluated the impacts of pipeline construction on hydraulic conductivity focused on saturated hydraulic conductivity, with some studies not specifying which hydraulic conductivity (saturated or near-saturated) they investigated. Our study investigated the impacts of underground pipeline construction on saturated and near-saturated hydraulic conductivity and how they change with increasing TSR. Results from this study indicated that both saturated and near-

saturated hydraulic conductivities were lower on the ROWs relative to adjacent off-ROW sites. This is consistent with results from other studies on reclaimed underground pipeline ROWs. For instance, in a quantitative synthesis of pipeline construction studies, Brehm and Culman (2022) reported an 11.2% mean decrease in hydraulic conductivity across six studies. In another study, hydraulic conductivity on the ROW was 10 times lower than on off-ROW sites (Soon et al., 2000). In these studies, the decrease in hydraulic conductivity on the ROWs was attributed to extensive soil compaction due to heavy construction vehicles and soil permeability alterations which occurred during pipeline construction and ROW reclamation activities. Although some studies were conducted on underground crude oil pipeline ROWs in grasslands and forest lands, their findings are directly comparable with underground natural gas pipeline ROWs on cropland since the installation processes of both pipeline types are basically similar. The lower K_{fs} and K_{ψ} on the 4.5-yr ROW relative to the off-ROW in our study are consistent with the PR measurements reported in Section 3.5.2 and reflect the influence of soil compaction on hydraulic conductivity. As with PR values, both saturated and near-saturated hydraulic conductivities showed a steady improvement with increasing TSR, indicating a progressive post-reclamation improvement in these properties and a possibility for both K_{fs} and K_{ψ} to recover within 12.5-yr following reclamation.

Saturated hydraulic conductivity is especially sensitive to soil compaction caused by field traffic (Dawidowski and Lerink, 1990; Soane and van Ouwerkerk, 1995). This is because soil compaction alters the total number, size, continuity, and geometry of soil pores (especially, the macropores, which are water-conductive under saturated conditions), resulting in reduced soil permeability (Watson & Luxmoore, 1986; van den Akker and Soane, 2005; Weil and Brady, 2017). These findings agree with those of Soon et al. (2000), who reported lower K_{fs} on the ROWs

than on adjacent undisturbed locations, which recovered with increasing TSR but was still substantially lower on the ROWs three years after reclamation. As in these studies, reduction in K_{fs} observed in our study could be due to soil structural collapse from underground pipeline construction activities, which reduced the number of macropores and disrupted pore continuity.

A reduction in near-saturated hydraulic conductivity is a big concern on cropland because it can result in increased surface water runoff and loss of nutrient-rich topsoil via surface soil erosion and reduced groundwater recharge due to restricted water infiltration and percolation (Zvomuya et al., 2009). In addition, it can also lead to reduced plant-available water in the root zone, and inadequate moisture to solubilize nutrients, all of which can lead to poor crop growth and performance (Cannon and Landsburg, 1990). Near-saturated hydraulic conductivity was still lower on the ROWs 6.5 yr after reclamation, indicating that the impacts of pipeline construction on soil water transport are long-lasting.

As hypothesized, both K_{ψ} and K_{fs} improved with increasing TSR. However, our results indicated that K_{ψ} recovered faster (took between 4.5 and 6.5 yr after reclamation) than K_{fs} , which took between 6.5 and 12.5 yr after reclamation to attain off-ROW levels. Recovery of physical properties on ROWs with increasing TSR is expected on cropped or vegetated landscapes due to the presence and activity of plants and repeated tillage activities. The addition of SOM from retained crop residues, the activity of crop roots as well as periodic tillage could all contribute to temporal improvements in hydraulic conductivity on the ROW with increasing TSR. Addition of organic amendments during reclamation of disturbed land or as a land management strategy during recovery has been reported to be an effective strategy for shortening the recovery period of disturbed land (Larney and Angers, 2012). This is because organic matter improves aggregate formation and stability, soil porosity, and biological activity, all of which enhance hydraulic

conductivity (Gasch et al., 2014; Larney and Angers, 2012). Furthermore, crop rotations such as those practiced at our field sites include crops of different root architectures, which create pores and crevices at different soil depths, thus aiding the recovery process (Spoor, 2006). Similarly, the periodic subsoiling of the fields may have contributed to reduced compaction and improved soil water movement over time, as has been observed elsewhere (Spoor, 2006).

3.5.4 Soil macropore indices

The volume and functional effectiveness of soil macropores can decrease when the soil is compacted (Drewry et al., 2008; Moraes et al., 2020). Therefore, the relatively low $\lambda_{\Delta\psi}$ and $N_{\Delta\psi}$ observed on the 4.5-yr ROW were conceivably due to soil compaction during pipeline construction. Moraes et al. (2020) also reported a decline in the size and number of macropores with increasing vehicular traffic in a crop field. The three porosity indices in our study showed a generally increasing trend with increasing TSR (except for a spike in $N_{\Delta\psi}$ on the 6.5-yr ROW, Table 3.3), which suggests a progressive recovery in these indices.

Our results show that effective water-conductive macroporosity ($\theta_{\Delta\psi}$), which describes the proportion of all macropores that remain water conductive at the investigated water supply pressure potentials (Zvomuya et al., 2009), accounted for a small proportion (<1%) of the total porosity of the soil at our study sites. This is consistent with observations from previous studies (e.g., Moret & Arr  e, 2007; Reynolds et al., 2008; Zvomuya et al., 2009). Effective macroporosity was lower on the 4.5-yr ROW than on the adjacent off-ROW locations and steadily improved with increasing TSR. The reduction in $\theta_{\Delta\psi}$ on the ROWs could be attributed to a structural decline in macroporosity and the number of effective water conductive macropores due to compaction-inducing pipeline construction activities. This observation agrees with other studies that looked at

the effects of soil compaction on hydraulic conductivity (Li et al., 2019; Moraes et al., 2020). For instance, heavy vehicular traffic reduced macroporosity by 92% in the surface layers of silty forest soils (Bottinelli et al., 2014). Similar to $\lambda_{\Delta\psi}$ and $N_{\Delta\psi}$, in our study $\theta_{\Delta\psi}$ improved with increasing TSR, which is likely due to the additive effects of natural attenuation processes and agronomic practices such as crop rotation, reduced tillage, periodic subsoiling, and addition of OM through crop residue retention. Incorporation of straw, sawdust and compost into a compacted clay loam soil was shown to improve saturated hydraulic conductivity by 15.9% to 34.4% (Eusufzai & Fujii, 2012). The authors attributed improvements in K_{fs} to increases in soil aggregation and macroporosity from amendment application. As such, it could be argued that crop residue incorporation enhanced the recovery of macroporosity, hence, K_{fs} , with increasing TSR in our study.

The trends observed for $\theta_{\Delta\psi}$ were consistent with those for PR, K_{fs} , and K_{ψ} , suggesting a close linkage among the four soil attributes. Soil macropores account for all water movement in saturated soils (K_{fs}) (Watson and Luxmoore, 1986; Weil and Brady, 2017). Consequently, extensive reduction in macroporosity due to soil compaction will decrease K_{fs} , as observed in this and other studies (e.g., Zhang et al., 2006; Soracco et al., 2011). However, in near-saturated soils, macropores are air-filled, and water conduction is predominantly via micropores (Weil and Brady, 2017). As such, K_{ψ} is characteristically high when the density of micropores is high. Soil compaction usually reduces macroporosity while microporosity increases or remains relatively unaffected (Weil and Brady, 2017). However, in this study, we assumed that extensive soil compaction on the ROWs also reduced microporosity, accounting for the low K_{ψ} observed on the 4.5-yr ROW.

3.5.5 Aggregate stability

Aggregate stability is of interest in cropping systems because it influences the soil's ability to support soil biological activity, root growth and development (penetration), resistance to erosion, water and nutrient retention, and nutrient availability for plant uptake (Aksakal et al., 2020; Li et al., 2019), all of which are critical for crop productivity. Aggregate formation and stabilization are mainly a function of biological and chemical factors, with soil organic matter playing a pivotal role in the processes (Weil and Brady, 2017). Many studies have shown low soil aggregation on disturbed soils and soils with low organic carbon, with aggregate stability showing a positive correlation with aggregate size distribution (Aksakal et al., 2020; Rieke et al., 2022). As a result, the low aggregate stability values observed on the 4.5-yr ROW could be due to a decline in soil structure from aggregate breakdown during pipeline construction, while improvements can be attributed to incorporation of crop residues. Rieke et al. (2022) attributed increased aggregate stability to increased OM from crop rotations, crop residue retention, and addition of organic amendments.

3.6 Conclusion

This study showed that underground pipeline construction activities impacted soil physical attributes (penetration resistance, saturated hydraulic conductivity, near-saturated hydraulic conductivity, and soil macroporosity) and SOC at the study sites. These impacts remained noticeable within the first 6.5 yr following reclamation of the ROWs for near-saturated hydraulic conductivity and SOC. The results also indicated that, with increasing time since reclamation, the impacts of pipeline construction tended to gradually disappear as soil physical attributes on the ROWs improved to levels similar to those on the adjacent off-ROW locations. Nonetheless, the

rate of recovery was not uniform across soil attributes. While penetration resistance, near-saturated hydraulic conductivity, and effective macroporosity appeared to have recovered within 6.5-yr post-reclamation, saturated hydraulic conductivity took between 6.5- and 12.5-yr to recover to off-ROW levels. The impact of pipeline construction activities on aggregate stability seemed minimal. This study is one of the first to explore macroporosity on reclaimed ROWs and show that underground pipeline construction impacts soil water transport through reduction in effective macroporosity. The soil physical properties measured in this study are known to influence crop productivity. Therefore, it is conceivable that crop yields on the ROWs would be reduced relative to adjacent off-ROW sections of the field and would recover gradually with increasing TSR. However, the extent and rate of yield recovery would also depend on chemical and biological factors, as well as other soil physical properties not tested in our study; these should be examined in future studies. In addition, we recommend including more ROWs (that is, greater replication) as well as more TSRs (i.e., younger and older ROWs than those included in our study) in future studies to sufficiently characterize the changes that occur to soil physical properties along a chronosequence of reclaimed ROWs.

3.7 References

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4. DYNAMICS OF SOIL MICROBIAL COMMUNITY DIVERSITY AND COMPOSITION ON UNDERGROUND NATURAL GAS PIPELINE RIGHTS-OF-WAY OF VARYING AGES

4.1 Abstract

Underground pipeline construction activities impact the soil ecosystem on pipeline rights-of-way (ROW). The structure and composition of soil microbial communities on reclaimed underground pipeline ROWs on cropland remains poorly understood despite the critical role of soil microorganisms in overall soil health. In this study, we examined changes in the diversity and composition of soil microbial communities with increasing time elapsed since reclamation of underground natural gas pipeline ROWs on cropland. Soil samples were collected from three sites in late summer of 2021 from the 0-15 cm layer on five ROWs with time since reclamation ranging from 4 to 12 yr and from adjacent undisturbed locations (off-ROW). At each site, soil samples were collected from the ROWs and the off-ROW. Metagenomic analyses of the 16S ribosomal RNA (rRNA) and the Internal Transcribed Spacer (ITS) rRNA genes were performed. The analysis involved steps of next-generation sequencing and clustering of reads (MiSeq Illumina sequencing), filtering, trimming, and chimeric removal of reads (rDNATools package), and operational taxonomic unit assignment (QIIME 2 platform). The results showed that (1) the diversity of bacterial and fungal communities was lower on the 5- and 6-yr ROWs than on the off-ROW at two out of the three sites and (2) diversity and abundance of some bacterial and fungal phyla improved with increasing time. These results suggest that bacterial and fungal communities impacted by pipeline construction would revert to pre-disturbance structure and composition in time.

Information generated from this study is crucial for formulating strategies to manage reclaimed ROWs for agricultural and environmental sustainability.

4.2 Introduction

Soil microorganisms perform various functions, which include decomposition of organic matter, nutrient cycling, soil energy transfer, mineralization of organic nutrients, stabilization of organic matter, aggregate formation, and protection of plant health by suppressing plant-pathogenic microbes and phytophagous fauna (Doran and Zeiss, 2000; Dangi et al., 2012; Hamidović et al., 2020). These ecosystem functions underscore the role of microorganisms in overall soil health. Soil microorganisms are found in greater concentration in the topsoil, in the Ah horizon, and close proximity to plant roots in the rhizosphere compared to the rest of the bulk soil (Gasch et al., 2016; Basiliko et al., 2021). The structure and composition of soil microbial communities correlate with soil properties such as pH, soil organic matter concentration, available nutrients, texture, and bulk density (Hamm et al., 2016; Wang et al., 2024; Xiang et al., 2024). As such, any activity that disturbs or changes topsoil attributes and the rhizosphere, such as uprooting vegetation, stripping and scrapping the topsoil, soil excavation, and mixing of the relatively fertile topsoil and the subsoil could potentially impact the structure, composition, and functionality of soil microorganisms.

The construction of underground pipelines for the transportation of natural gas involves all or some of the activities listed above. Impacts of pipeline construction on soil microbial communities are also expected from vehicle-induced compaction on ROWs. Compaction reduces the size and number of soil macropores which are critical for soil aeration (Tekeste et al., 2019). Thus, highly compacted soils, such as those on ROWs, are poorly aerated. This could negatively impact the diversity and abundance of aerobic microorganisms. In addition to changes to the soil

ecosystem due to the construction activities, the physical presence of an operating pipeline with its pressurized contents also creates a microenvironment of elevated soil temperature and reduced soil moisture in the vicinity of the pipeline relative to the rest of the bulk soil (Naeth et al., 1993; Halmová et al., 2017). For instance, Shi et al. (2015) showed a 1 to 2 °C increase in soil temperature on reclaimed underground pipeline rights-of-way (ROWs) compared to the adjacent undisturbed locations (off-ROW). Studies have shown that soil microorganisms are generally sensitive to changes in land use (Wipf et al., 2020; Chen et al., 2021; Esmaeilzadeh-Salestani et al., 2021) and to land degradation involving excavation (Li et al., 2015; Hou et al., 2018; Hamidović et al., 2020). Due to these factors, the disruption of microbial communities on reclaimed underground pipeline ROWs is highly probable. However, studies on reclaimed sites showed that, in most cases, microbial communities tend to recover to pre-disturbance levels with increasing time post-reclamation (Hou et al., 2018; Qiang et al., 2020).

Despite increasing reports on the potential impacts of underground pipeline construction on soil microbial communities, the effect of time elapsed since ROW reclamation on soil microbial communities has not been widely investigated (Brehm & Culman, 2022). Studies that evaluated the effects of underground pipeline construction on microbial properties used techniques such as microbial biomass carbon (Soon et al., 2000), permanganate-oxidizable carbon (Schindelbeck & van Es, 2012), and phospholipid fatty acids analysis (Gasch et al., 2016). Although these techniques are effective in quantifying soil microbial activity, they are limited in their resolution because they only measure the general combined activity of soil microorganisms without identifying individual microorganisms. For instance, microbial biomass carbon provides an estimate of overall soil biological activity, which is the combined activity of bacterial, fungal, and archaeal microorganisms but does not distinguish among the soil microorganisms (Doran and

Parkin, 1994). Although, the phospholipid fatty acids analysis technique has a better resolution than microbial biomass carbon and permanganate-oxidizable carbon; it can only distinguish microorganisms to the kingdom level (Gasch et al., 2016). On the other hand, nucleic acid techniques, such as 16S and Internal Transcribed Spacer (ITS) ribosomal ribonucleic acid (rRNA) gene sequencing can provide a higher taxonomic resolution down to the species level and can detect soil microorganisms present in very low numbers, something that microbial biomass carbon cannot achieve (Johnson et al., 2019). Thus, higher resolution techniques such as 16S gene sequencing could provide greater insights into the changes in soil microbial populations due to anthropogenic activities compared to the proxy methods such as microbial biomass carbon and phospholipid fatty acid analysis.

Our study is among the first to investigate the impacts of pipeline construction on bacterial and fungal communities and the changes thereof with increasing time since reclamation using nucleic acid analysis techniques (16S and ITS rRNA gene sequencing). Given the importance of soil microorganisms in soil productivity and the existing knowledge gaps, the overall objective of this study was to evaluate the effect of time elapsed since the reclamation of natural gas ROWs on the diversity and structure of soil bacterial and fungal communities. We hypothesized that underground pipeline construction would reduce soil microbial diversity and abundance on reclaimed ROWs and that diversity and abundance of soil bacteria and fungi would increase with increasing time elapsed since reclamation. The information generated from this study is critical to land reclamation specialists in devising strategies to restore reclaimed ROWs to pre-disturbance capability.

4.3. Materials and methods

4.3.1 Description of study area

Soil samples were collected from natural gas pipeline ROWs on cropland in the Farmington area (55° 54' N; 120° 30' W) in northeastern British Columbia, Canada (Figure 4.1). The long-term (1981-2010) mean temperature for the area is 13.02 °C during the growing season (May to September) and -10.1 °C during winter (November to February). The mean annual precipitation is 445 mm (Environment and Climate Change Canada, 2023). The dominant soils at the study sites are Orthic Gray Luvisols in the Canadian Soil Classification System (Typic Cryoboralf) with a silt loam texture and an acidic-to-neutral pH (B.C. Soil Information Finder Tool, 2018). The soil samples were collected from three sites. Site 1 is at an elevation of 757.7 m above mean sea level (amsl) with an average slope of 2.6%, site 2 is at an elevation of 766.3 m amsl with an average slope of 2.3%, and site 3 is at an elevation of 765.4 m amsl with an average slope of 2.5% (Google Earth Pro, 2024). Because of its climatic and soil characteristics (Canada Land Inventory Classes 2-4) (B.C. Soil Information Finder Tool, 2018), the region falls under British Columbia's Agricultural land reserve (B.C. Agricultural Land Commission, 2022) and accounts for one-third of the land reserve. The Agricultural land reserve (~ 4.7 million ha, or 4.9% of British Columbia's land area) is the provincial zone established to protect agricultural land and in which agriculture is the priority land use, with non-agricultural uses restricted (B.C. Agricultural Land Commission Act, 2019). Canada Land Inventory Classes 1-4 lands are generally considered the key agricultural lands of British Columbia and are included within the province's land reserve, although forage lands (Class 5) and spring and fall grazing lands (Class 6) are also included. Agricultural activities in the study area include livestock and crop production, with the major crops being canola (*Brassica rapa* L.), spring wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena*

sativa L.), field pea (*Pisum sativum* L.), and forages. Agronomic practices at the study sites include crop rotations and crop residue retention with harrowing to even out crop stubbles. The fields are also subject to periodic sub-soiling and reduced tillage using zero-till drills with hoe-type openers for seeding. The region is also rich in oil and natural gas resources. Accommodation of the oil and gas activities on the land reserve was based on the understanding that agricultural land disturbed by activities such as underground pipeline construction would be restored, through reclamation, to the productivity that existed prior to the disturbance (B.C. Agricultural Land Commission, 2012).

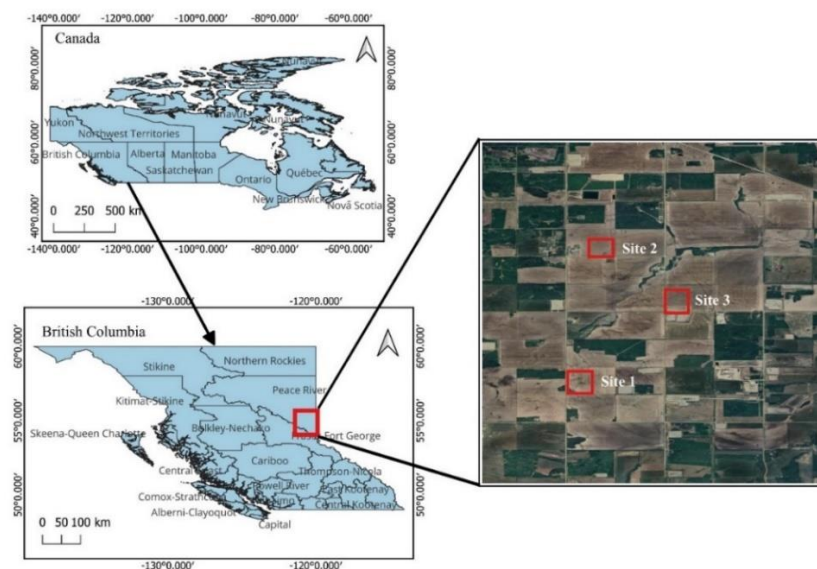


Figure 4.1 Location of the study area and sites in the Farmington area, BC, Canada.

4.3.2 Treatment description

The soil treatments at each of the three study sites consisted of ROWs of different ages (i.e., time since reclamation, TSR) and the adjacent undisturbed locations (off-ROW): Site 1, 6- and 12-year ROWs and the off-ROW; Site 2, 5- and 11-year ROWs and the off-ROW; and Site 3, 4-year ROW and the off-ROW. Soil microbial communities are highly variable even across short

distances in one field; thus, variability across fields (sites) is expected to be high due to differences in soil properties.

4.3.3 Soil sampling procedure

Soil samples were collected at the time of crop harvesting in September 2021 using a hand-held auger from the 0–15 cm soil layer on natural gas pipeline ROWs of varying TSR and the off-ROW across the three sites. The three sites sampled in this study were different fields within a radius of ~5 km. In the year of sampling, Sites 1 and 2 were cropped with wheat, while Site 3 was cropped with barley. On each site, three sampling locations (4 m² each) were marked on each of the ROWs and on the off-ROWs. The sampling locations on each treatment were 25 m apart. The off-ROW sampling locations were 50 m away from the ROWs. Studies have shown that soil attributes 50 m away from the ROWs are not significantly impacted by pipeline construction and, therefore, could be used as benchmark sites (Desserud et al., 2010; Shi et al., 2015). Four random cores were collected from each sampling location, placed into a clean polythene bag, and thoroughly mixed by shaking the polythene bag and breaking large soil clods by hand to make one composite sample. Two subsamples of approximately 200 g each were then taken from the composite sample. One of the subsamples was placed into a clean polythene bag and stored in a cooler box with ice blocks for transportation to the Department of Soil Science, University of Manitoba where the samples were stored at -80 °C until DNA extraction and metagenomic analysis. The other sample was placed into a separate clean polythene bag and transported in a cool container to the Department of Soil Science, University of Manitoba where the samples were stored at 4 °C until they were processed for chemical properties analysis.

4.3.4 Soil chemical properties analysis

Soil organic matter (SOM) was measured using the loss on ignition (360 °C) method (Combs & Nathan, 2015). Total carbon (TC) was determined using the dry combustion method (Nelson and Sommers, 1996). Inorganic carbon (IC) was estimated by pressure calcimetry after reacting the soil with 15% HCl (Loeppert and Suarez, 1996). Soil organic carbon (SOC) was calculated as TC minus IC (Nelson and Sommers, 1996). Electrical conductivity (EC) and soil pH were measured using an EC meter (Accumet® Basic, AB30) and a pH meter (Accumet® Basic, AB15), respectively, in a 1:2 soil to deionized water paste. Nitrate-N was measured colorimetrically following reduction with copperized cadmium (Gelderman & Beegle, 2015). Available P (Olsen-P) was determined by the ascorbic acid-ammonium molybdate method (Murphy and Riley, 1962) following extraction (1:10 soil to solution ratio) with 0.5 M sodium bicarbonate at a pH of 8.5 (Olsen et al., 1954). Sulfate S was extracted with 0.12 M KCl and determined by turbidimetry (Franzen, 2015). Potassium (K), calcium (Ca), and magnesium (Mg) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Warnacke and Brown, 2015).

4.3.5. DNA extraction, PCR amplification, and Illumina sequencing

Twenty-four frozen soil samples (three replicates per treatment) were shipped to Psomagen, Rockville, USA, for DNA extraction, amplicon library creation, and metagenome amplicon sequencing. Microbial genomic DNA was extracted from the soil samples using the Qiagen DNeasy® PowerSoil® Pro Kit following the manufacturer's protocol (Qiagen, Hilden, Germany). The concentration of the extracted DNA was quantified using the Picogreen® method (Thermo Fisher Scientific, Waltham, MA, USA). The quality of the extracted DNA was assessed using the

Agilent Genomic DNA ScreenTape system (Agilent Technologies, Santa Clara, CA, USA). The gel electrophoresis of DNA samples confirmed the presence of intact genomic DNA with a prominent band at approximately 10 kb. The DNA was amplified by PCR for downstream sequencing targeting the 16S rRNA V3-V4 region in bacteria using the primer set 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC) (Herlemann et al., 2011). For fungi, the ITS rRNA gene was targeted using the primer set ITS3F (GCATCGATGAAGAACGCAGC) and ITS4R (TCCTCCGCTTATTGATATGC) (White et al., 1990). Metagenome amplicon sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA).

4.3.6 Processing and analysis of bioinformatic data

The raw data generated by the Illumina MiSeq platform were demultiplexed with index sequences. The FASTP program was used to trim adapter sequences and correct errors due to overlapping reads. The target sequences were assembled with FLASH (Fast Length Adjustment of Short reads) (v1.2.11) by merging paired-end sequences created by sequencing of both directions of the library. Among the assembled reads, only reads, which were > 300 bp and < 500 bp for fungi and > 400 bp and < 500 bp for bacteria, were filtered by length. Sequence pre-processing to remove noise data (sequences with low quality) and Operational Taxonomic Unit (OTU) clustering of the remaining reads using a cluster cutoff value of 97% were done using CD-HIT-OTU-MiSeq approach. Taxonomic classification for each sample from phylum to species levels was performed with QIIME - UCLUST program using the RDP reference database for 16S rRNA gene and UNITE reference database for the ITS gene. Community diversity analyses were conducted using the QIIME (v1.9.0) program. Microbial community richness and diversity (Alpha diversity) was evaluated using the Chao1 (Eq. [4.1]), Shannon (Eq. [4.2]), and Simpson indices. The Simpson index was calculated as $1 - \text{Gini-Simpson index}$.

$$S_{chao1} = S_{obs} + \frac{n1(n1-1)}{2(n2+1)} \quad [4.1]$$

where S_{chao1} is the estimated number of OTUs, S_{obs} is the observed number of OTUs, $n1$ is the estimated number of OTUs that contained only one sequence, and $n2$ is the number of OTUs that contained two sequences.

$$H_{shannon} = - \sum_{i=1}^{S_{obs}} \frac{n_i}{N} \ln \frac{n_i}{N} \quad [4.2]$$

where S_{obs} is the observed number of OTUs, n_i is the number of sequences contained in the i th OTU, and N is the total number of sequences.

4.3.7 Statistical analysis

Data were analyzed in SAS® OnDemand for Academics (SAS Institute Inc, 2024). Data were subjected to one-way analysis of variance (ANOVA) using the generalized linear mixed model procedure (PROC GLIMMIX) to determine treatment effects on bacterial and fungal community attributes. The Poisson distribution was used for OTU and Chao1 data, while the beta distribution was used for Simpson, and phyla relative abundance data. Treatment means were compared using Tukey's multiple comparison procedure at $\alpha = 0.05$. Pearson correlation analysis was conducted to determine the relationship between soil microbial community variables and other soil attributes.

4.4. Results

4.4.1 Soil chemical properties

Soil organic C concentration was lower on the ROWs than on the off-ROWs at Sites 1 and 3 (Table 4.1). On Site 1, SOC was 29% and 20% lower on the 6- and 12-yr ROWs, respectively, than on the off-ROW, while on Site 3 it was 27% lower on the 4-yr ROW than on the off-ROW.

Significant ($p = 0.0001$) treatment effects for $\text{NO}_3\text{-N}$ and Olsen-P concentrations were detected on Site 2 only, where the 11-yr ROW had significantly lower $\text{NO}_3\text{-N}$ concentration than the other treatments and the off-ROW had significantly lower Olsen-P concentration than the ROWs. Extractable K concentration was 31.3 % lower ($p = 0.02$) on the 4-yr ROW than on the off-ROW. On Site 1, extractable Ca concentration was significantly ($p = 0.003$) greater on the ROWs than on the off-ROW. On site 2, Ca was 104% greater on the 5-yr ROW and 27% greater on the 11-yr ROW than on the off-ROW. On Sites 2 and 3, extractable Mg concentration was significantly greater on the ROWs than on the off-ROW. Across all treatments and sites, soil pH was mostly neutral, ranging from 6.8 to 7.1. On Site 2, EC was 58.5% greater on the 5-yr ROW than on the off-ROW.

Table 4.1 Selected soil chemical properties on ROWs and off-ROWs.

Site	TSR†	SOC g kg ⁻¹	NO ₃ -N	Olsen-P	K	Ca mg kg ⁻¹	Mg	pH	EC mS cm ⁻¹
Site 1	6 yr	25 b‡	26.1 a	17.2 a	85.6 a	1777 a	331 a	7.0 a	0.30 a
	12 yr	28 b	26.0 a	14.4 a	80.7 a	1827 a	259 a	6.8 a	0.37 a
	Off-ROW	35 a	31.6 a	18.4 a	90.3 a	1487 b	255 a	6.9 a	0.38 a
	<i>P-value</i>	0.003	0.41	0.34	0.61	0.003	0.23	0.06	0.33
Site 2	5 yr	35 a	51.1 a	31.7 a	106 a	2870 a	557 a	6.8 a	0.17 a
	11 yr	24 b	22.6 b	27.4 a	108 a	1786 b	266 b	7.1 a	0.42 b
	Off-ROW	21 b	66.1 a	17.4 b	112 a	1404 c	174 c	7.0 a	0.41 b
	<i>P-value</i>	0.0001	0.0001	0.0013	0.23	0.0002	0.0001	0.32	0.0001
Site 3	4 yr	22 b	51.8 a	30.2 a	106 b	1340 a	217 a	7.0 a	0.17 a
	Off-ROW	30 a	42.8 a	33.6 a	154 a	1350 a	158 b	7.1 a	0.30 a
	<i>P-value</i>	0.001	0.58	0.46	0.02	0.95	0.01	0.42	0.11

†TSR, time since reclamation; SOC, soil organic carbon; NO₃-N, nitrate nitrogen; K, available potassium; Ca, extractable calcium; Mg, extractable magnesium; EC, electrical conductivity

‡Means in a column within the same site followed by the same letter are not significantly different at $\alpha = 0.05$ according to Tukey's multiple comparison procedure

4.4.2 Diversity of soil bacterial and fungal communities

OTUs ranged from 499 to 818 for bacteria and from 368 to 496 for fungi (Table 4.2). The diversity of both bacterial and fungal communities was site-dependent (Table 4.2). There were no significant differences in bacterial and fungal diversity indices on Site 3 among the ROWs and between the ROWs and the off-ROWs. However, the Chao1 index indicated that bacterial diversity on Site 1 was 18.2% lower on the 6-yr ROW than on the off-ROW while the 12-yr ROW had similar diversity to the off-ROW, suggesting a significant improvement in bacterial diversity with increasing TSR. On Site 2, the Chao1 bacterial diversity index was 15.3% lower ($p = 0.001$) on the 5-yr ROW than on the off-ROW. The TSR effect on the Simpson diversity index for bacteria was significant only on Site 2, where the 11-yr ROW showed the lowest Simpson diversity index while the 5-yr ROW was similar to the off-ROW.

For fungi, Chao1 diversity was 15.4% lower ($p = 0.01$) on the 6-yr ROW than on the off-ROW on Site 1 while it was 16.3% lower ($p = 0.001$) on the 5-yr ROW than on the off-ROW on Site 2 (Table 4.2). On Site 1, fungal community diversity had not recovered by year 12 following ROW reclamation while on Site 2 recovery to off-ROW levels took 11 yr.

Table 4.2 Diversity of bacterial and fungal gene sequences in soils collected from reclaimed underground pipeline right-of-ways of varying ages

Site	TSR†	OTU		Chao1		Shannon		Simpson	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
Site 1	6 yr	638 b‡	436 a	778 b	476 b	8.01 a	6.26 a	0.0086 a	0.0326 a
	12 yr	757 a	446 a	884 a	502 b	8.23 a	6.13 a	0.0077 a	0.0437 a
	Off-ROW	818 a	496 a	951 a	562 a	8.19 a	6.18 a	0.0085 a	0.0447 a
	<i>P-value</i>	0.001	0.14	0.001	0.01	0.07	0.9	0.51	0.59
Site 2	5 yr	544 b	387 b	683 b	436 b	7.49 a	4.60 b	0.0167 a	0.1599 a
	11 yr	507 b	480 a	641 b	562 a	7.78 a	6.30 a	0.0108 b	0.0387 b
	Off-ROW	648 a	455 a	807 a	522 a	7.66 a	6.39 a	0.0146 ab	0.0348 b
	<i>P-value</i>	0.001	0.03	0.001	0.001	0.29	0.03	0.016	0.005
Site 3	4 yr	499 a	368 a	608 a	425 a	7.25 a	5.62 a	0.0145 a	0.0580 a
	Off-ROW	518 a	395 a	615 a	451 a	7.45 a	6.00 a	0.0129 a	0.0329 a
	<i>P-value</i>	0.36	0.16	0.75	0.96	0.59	0.33	0.7	0.15

†TSR, time since reclamation; OTU, operational taxonomic unit

‡Means in a column within the same site followed by the same letter are not significantly different at $\alpha = 0.05$ according to Tukey's multiple comparison procedure

4.4.3 Microbial community composition

A total of 131,082 sequences of sufficient quality were obtained from the 24 soil samples. For bacteria sequences ranging from 1,856 to 9,567 per sample (mean = 5,461) were obtained while for fungi, 437,675 sequences of sufficient quality, ranging from 12,294 to 23,975 (mean = 18,236) per sample were obtained. The Good's coverage ranged from 92.0% to 98.9% for bacteria and 99.3% to 99.8% for fungi, indicating that the depth of this sequencing reflected the actual bacterial and fungal communities in the soil samples.

Across ROWs/off-ROWs and sites, 11 abundant (> 1% relative abundance) bacterial phyla were identified in the soil samples (Figure 4.1a and Table S4.1). The phyla and their relative abundances were Acidobacteria (7.0-16.4%), Actinobacteria (23.8-31.6%), Bacteroidetes (2.3-7.8%), Chloroflexi (2.6-5.2%), Gemmatimonadetes (3.6-5.4%), Planctomycetes (2.8-4.6%), Proteobacteria (2.1-21.5%), Verrucomicrobia (3.1-12.9%), Firmicutes (3.2-12.4%), Nitrospirae (0.1-1.4%), and WPS-1 (0.6-1.8%). Acidobacteria were significantly lower on the 6-yr ROW (11.4%) than on the off-ROW (16.4%) on Site 1 and lower on the 5-yr ROW (7%) than on the off-ROW (14.9%) on Site 2. The relative abundance of Actinobacteria did not show any consistent trend across the sites and ROWs/off-ROWs. The relative abundance of Bacteroidetes was significantly greater on the 5-yr ROW (7.8%) than on the off-ROW (2.3%) on Site 2 only. Chloroflexi were significantly lower on the 6-yr ROW (3.2%) than on the off-ROW (4.6%) on Site 1 and on the 4-yr ROW (2.6%) than on the off-ROW (5.2%) on Site 3. The relative abundance of Gemmatimonadetes was significantly lower on the 6-yr ROW (4.7%) than on the off-ROW (5.3%) on Site 1 only. The TSR effects were significant for Firmicutes on Site 3 where the relative abundance was greater on the 5-yr ROW (12.4%) than on the off-ROW (3.6%). The abundance of Verrucomicrobia was significantly lower on the ROWs than on the off-ROW on Site 2 only. For

Nitrospirae, relative abundance was significantly greater on the off-ROW (1.3%) than on the 6-yr ROW (0.7%) on Site 1 and on the off-ROW (1.4%) than on the 5-yr ROW (0.5%) on Site 2. The relative abundance of Acidobacteria, Chloroflexi, Gemmatimonadetes, Proteobacteria, Verrucomicrobia, Nitrospirae showed an improvement with increasing TSR on two sites while for Bacteroidetes and Firmicutes it was greater on the 5-yr ROW than on the off-ROW on Site 2.

Eighteen bacterial genera with relative abundance > 1% on at least one ROW/off-ROW were identified across the sites and treatments (Figure 4.1b). The relative abundance of bacteria at the genus level did not show a consistent pattern across the treatments and sites. Overall, the most abundant genera were *Spartobacteria genera incertae sedis* (22.9%), *Gp6* (23.1%), *Gemmatimonas* (21.4%), *Bradyrhizobium* (17.1%), and *Gaiella* (22.7%).

The most abundant fungal phyla across sites and TSR treatments were Ascomycota (40.3-72.2%), Basidiomycota (1.0-4.7%), Monoblepharomycota (0.01-0.1%), Mortierellomycota (0.9-5.0%), and Mucoromycota (0.1-5.9%) (Figure 4.2a; Table S4.2). Significant TSR effects on the relative abundance of fungal phyla were detected on Sites 2 and 3 only for Ascomycota, Basidiomycota, and Mucoromycota. On Site 2, the relative abundance of Ascomycota was significantly greater ($p = 0.003$) in soil from the 5-yr ROW (72.2%) than that from the 11-yr ROW (57.4%) and the off-ROW (53.6%) while the 11-yr ROW was similar to the off-ROW. Basidiomycota were significantly lower ($p = 0.01$) on for the 5-yr ROW (1%) than the 11-yr ROW (3.8%) and the off-ROW (2.2%) on Site 2 while the 11-yr ROW and the off-ROW did not differ significantly on Site 2. The relative abundance of Mucoromycota on Site 2 was significantly ($p = 0.03$) greater on the 5-yr ROW (1.5%) than on the off-ROW (0.3%). Similarly, on site 3, the relative abundance of Mucoromycota was greater on the 4-yr ROW (0.3%) than on the off-ROW (0.1%). The most abundant fungal genera were *Mortierella* (18.7%), *Mucor* (16.9%),

Solicoccozyma (7.1%), *Exophiala* (5.4%), and *Penicillium* (3.4%). However, response of the relative abundance of the fungal genera to TSR did not show a consistent pattern (Figure 4.2b).

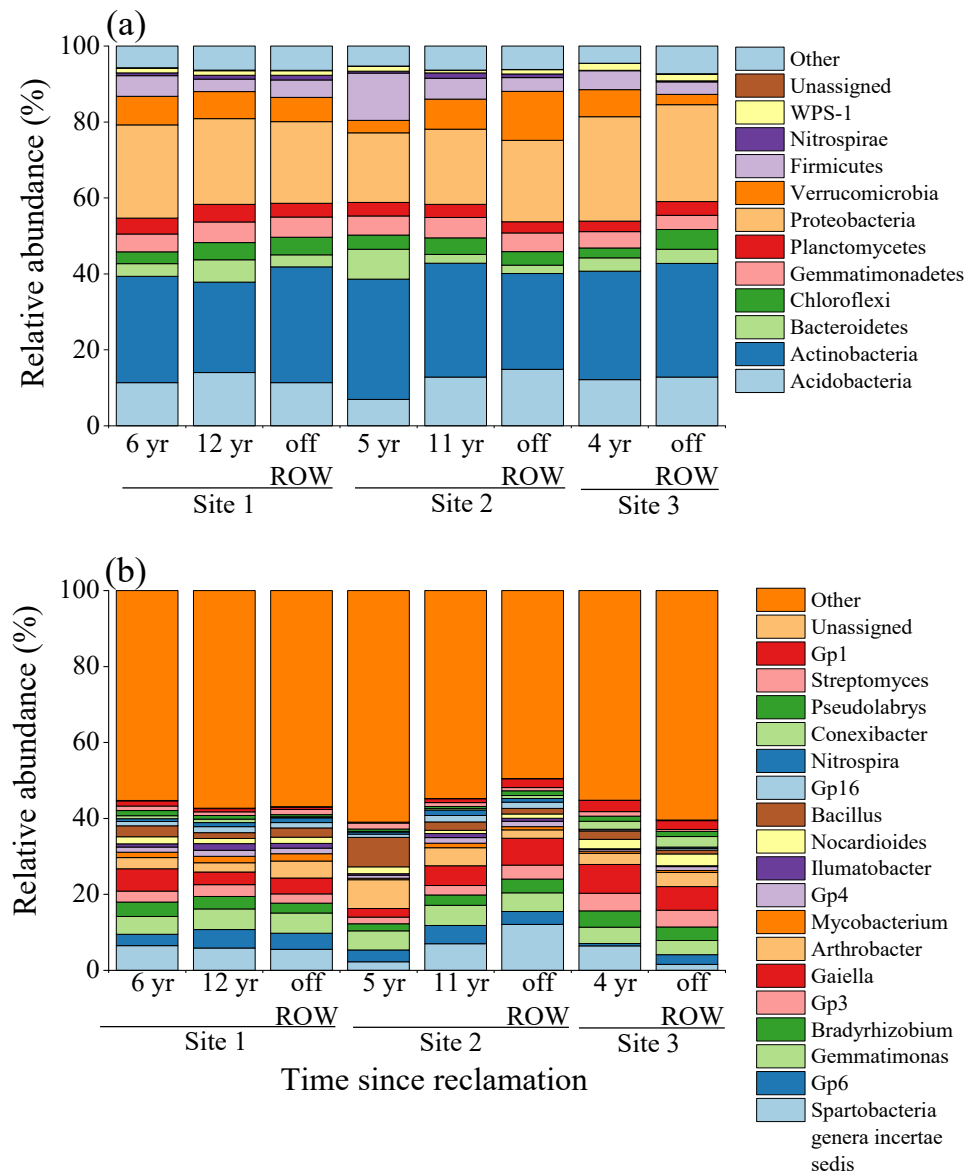


Figure 4.2 Relative abundance of bacterial (a) phyla and (b) genera with relative abundance greater than 1% in all treatments. Phyla and genera with relative abundance less than 1% in all treatments were classified under 'other'.

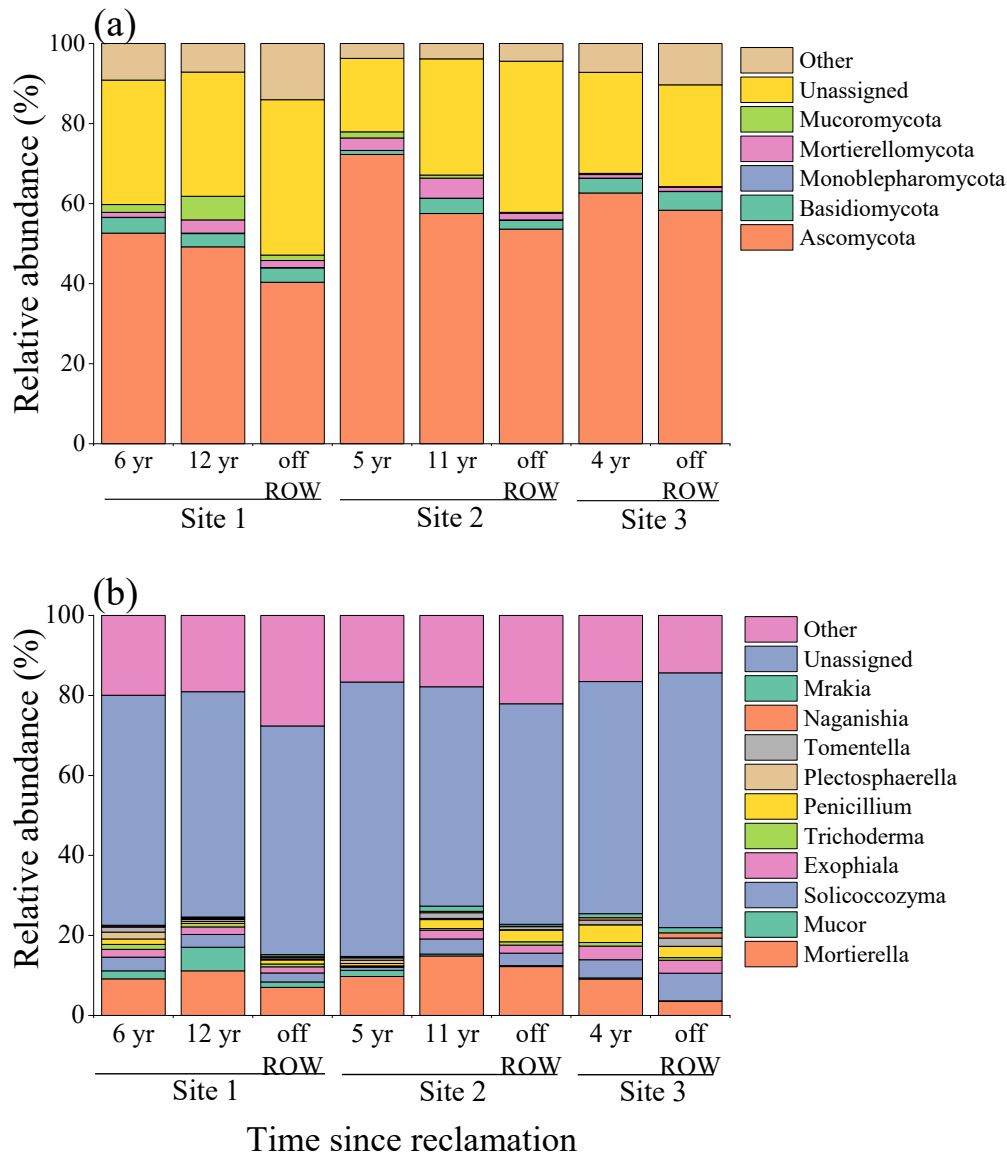


Figure 4.3 Relative abundance of fungal (a) phyla and (b) genera with relative abundance greater than 1% in all treatments. Phyla and genera with relative abundance less than 1% in all treatments were classified under ‘other’.

4.4.4 Relationship between soil microbial communities and other soil attributes

Pearson’s correlation analysis showed significant relationships between some soil attributes and some microbial characteristics (Figure 4.4 and Table S4.3). The Chao1 index for

bacteria was negatively correlated with Olsen-P and EC, while the Shannon index (bacteria) was negatively correlated with NO₃-N, Olsen-P, and available K. The Chao1 index (fungi) was negatively correlated with Olsen-P and available K. The Shannon index (fungi) was negatively correlated with TOC, Olsen-P, available K, Ca, and Mg. Ascomycota was positively correlated with NO₃-N, Olsen-P, available K, Ca, and Mg, while Basidiomycota was negatively correlated with available K, Ca, Mg, and EC. Actinobacteria were positively correlated with TOC, Olsen-P, and available K. Firmicutes showed a positive correlation with TOC, available K, Ca, and Mg. The rest of the abundant bacterial phyla showed negative correlations with soil properties.

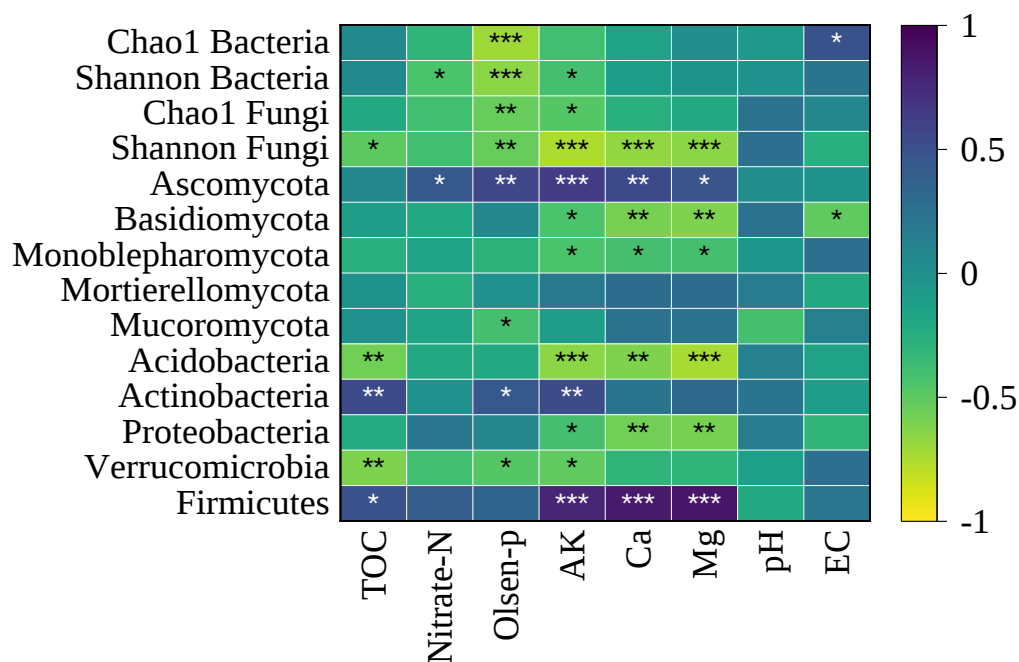


Figure 4.4 Correlation analysis between soil properties and microbial attributes. TOC, total organic carbon; Olsen-P, available phosphorus; AK, available potassium; EC, electrical conductivity. Asterisks indicate significant correlation at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

4.5 Discussion

4.5.1 Soil chemical properties

Our results showed that SOC was lower on reclaimed ROWs than on the off-ROW on two out of three sites. These results suggest that underground pipeline construction activities could have negatively impacted SOC concentration in the soil. Reduced SOC on the ROWs than on the off-ROW could be attributed to pipeline construction activities such as topsoil scrapping and soil excavation. In addition, poor crop growth and yield on reclaimed ROWs relative to the off-ROW also result in less SOM inputs on reclaimed ROWs than on the off-ROW. In our complimentary study (Chapter 2), crop biomass yields on the ROWs were 36-57 % lower than on the off-ROW. However, the cumulative SOM addition of crop residues with increasing TSR could significantly improve SOC; hence, the observed SOC increase with increasing TSR. The topsoil is relatively rich in SOM compared to the subsoil (Larney & Angers, 2012). Therefore, a significant reduction in SOC on reclaimed ROWs could be expected if the topsoil is scrapped and not sufficiently salvaged and backfilled. Reduction in SOC concentration due to pipeline construction and its improvement with increasing TSR could influence the structure and function of soil microbial communities on reclaimed ROWs since soil C is a major energy source for soil microorganisms.

4.5.2 Microbial community diversity

To our knowledge, this is the first study to examine the impact of underground pipeline construction on the soil microbiome and its recovery over time using genomic techniques (16S rRNA and ITS sequencing and analysis). Previous studies have used other indirect methods, such as microbial biomass carbon (MBC) (Soon, Rice, et al., 2000), permanganate oxidizable carbon (POXC) (Schindelbeck & van Es, 2012), and phospholipid fatty acids (PLFAs) (Gasch et al.,

2016). Those studies showed variable results, possibly due to the generalizations provided by these methods compared to genomic techniques, which are relatively precise. For instance, using MBC to evaluate the impacts of pipeline construction on soil biological properties of boreal soils on cropland in Alberta, Canada, Soon, et al. (2000) found inconsistent year-to-year results and concluded that, overall, the MBC was adversely impacted by pipeline construction.

In our study, bacterial and fungal Chao1 values were lower on the youngest pipeline ROWs than on the off-ROWs on two of the three sites, suggesting that underground pipeline construction activities had deleterious effects on some soil bacterial and fungal species on the ROWs on these sites. In addition, on these two sites, the Chao1 values improved as the TSR increased from 6 to 12 on Site 1 and from 5 to 11 yr on Site 2. This indicated an improvement in bacterial and fungal community diversity on the reclaimed ROWs with increasing TSR, which corroborated our hypotheses. In general, anthropogenic activities that involve vegetation removal, soil excavation, and soil piling for extended periods have been shown to reduce soil microbial diversity (e.g., Li et al., 2014; Li et al., 2018; Ezeokoli et al., 2020). This is because, compared to the bulk soil, microorganisms are more concentrated in the rhizosphere, where some of them form symbiotic relationships with plants, thus uprooting the plants disrupts this microhabitat for soil microorganisms (Basiliko et al., 2021).

The greatest impact of pipeline construction on the soil ecological system is expected to come from soil excavation and soil handling during salvaging and backfilling (Schindelbeck & van Es, 2012). Soil excavation alters soil physicochemical properties such as soil structure, soil pH, SOM, and SOC, which are key drivers of the microbiome structure and microbial activities (Banerjee & van der Heijden, 2023; Philippot et al., 2024). As mentioned earlier, previous studies showed minimal impacts of pipeline construction on microbial communities (e.g., Soon et al.,

2000; Gasch et al., 2016;). However, it is noteworthy that the methods used in these studies did not distinguish between diversity and abundance of soil microorganisms. In addition, the studies were conducted on non-cropland except for Soon et al. (2000). However, our results are consistent with those of Schindelbeck & van Es (2012), who showed a significant decrease in POXC (an indirect indicator of microbial activity) on ROWs relative to off-ROW sites across several land use types, including cropland. The authors attributed the decline in POXC to soil horizon mixing. Our study sites showed no significant mixing of horizons (Muitire et al., 2024).

Time since reclamation has been reported to influence the recovery of microbial communities on sites disturbed by soil excavation on landscapes such as estuaries (Sun et al., 2011) and mining sites (Li et al., 2014). In these studies, the improvement in soil microbial diversity with time was attributed to the natural attenuation of soil attributes as the soil builds itself over time. The improvement in soil microbial diversity with increasing TSR observed in our study could be due to the combined effects of natural soil-building processes and agricultural practices. The pipeline ROWs in this study were constructed on croplands where crop rotations, reduced tillage, and crop residue retention are practised. Cropping systems, such as crop rotations, that encourage the build-up of organic carbon have been shown to promote soil microbial diversity on cropland (Esmaeilzadeh-Salestani et al., 2021). Plant roots create favorable conditions for the growth and activity of soil microorganisms by penetrating deep into the soil, thus loosening and aerating the soil (Wang et al., 2024). Additionally, plants, through their roots and above-ground biomass, add substantial organic matter to the soil, which aids the recovery of the reclaimed ROWs with increasing TSR and stimulates the proliferation of soil microorganisms by providing an energy source (Wang et al., 2024). Our results presented in Section 4.4.1 showed a significant increase in SOC with increasing TSR, which we attributed to the addition of crop residues.

4.5.3 Microbial community composition

Understanding the community structure of soil microorganisms on disturbed and reclaimed landscapes is crucial to understanding the processes and drivers of the recovery from a disturbance. This information can help in formulating effective techniques to accelerate the recovery process by intentionally altering the soil ecosystem to stimulate the proliferation of certain beneficial soil microorganisms. While 16S rRNA and ITS techniques have not been widely used to study microbial communities on cropland disturbed by pipeline construction, these techniques have been widely used to evaluate the recovery of reclaimed mining sites (e.g., Dangi et al., 2012; Li et al., 2014; Li et al., 2016) and to examine the effect of different agronomic practices on soil microbial communities (e.g., Souza et al., 2013; Rao et al., 2021; Yang et al., 2021). Although mining sites are different from pipeline ROWs, the response of microorganisms might be the same if no contaminants are involved.

Similar to microbial community diversity, the response of bacterial and fungal community composition varied among sites, reflecting the influence of site characteristics on the resilience of the soil ecosystem to anthropogenic disturbances and its capacity to recover to pre-disturbance levels. However, the TSR effects were more pronounced at the phylum level than at the genus level for both bacteria and fungi. Across study sites and treatments, abundant bacterial phyla were Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Planctomycetes, Proteobacteria, Verrucomicrobia, Firmicutes, Nitrospirae, and WPS-1. This soil bacterial phylum-level profile is consistent with those previously reported for other soils and environments (e.g., Esmaeilzadeh-Salestani et al., 2021; Rao et al., 2021; Wang et al., 2024). In general, members of the Proteobacteria and Acidobacteria phyla are ubiquitous in almost all soil types across the globe (Zhang & Xu, 2008). In our study, the fungal community was dominated by the phyla Ascomycota,

Mortierellomycota, and Basidiomycota across all sites and treatments. These fungal phyla are commonly found in the topsoil of different soils in different environments, with Ascomycota dominating fungal communities globally (Egidi et al., 2019; Banerjee & van der Heijden, 2023).

Our results showed that the relative abundances of some bacterial phyla (Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Proteobacteria, and Nitrospirae) and Basidiomycota improved significantly with increasing TSR on at least one of the three sites and were similar to those on the off-ROWs after 11-12 yr post-reclamation, thus supporting our hypotheses. Previous studies also showed an increase in the relative abundance of Proteobacteria, Acidobacteria, and Actinobacteria with increasing TSRs in lands disturbed by various anthropogenic activities, which was attributed to improvements in plant cover and soil chemical attributes with time (Li et al., 2016; Yang et al., 2021; Bonomo et al., 2022). In our study, there was an improvement in SOC with increasing TSR, which could have resulted in the observed improvement in the relative abundance of bacteria and fungi. Soil physicochemical attributes significantly influence microbial community structure and functionality by providing energy sources and a conducive environment for microbial proliferation (Singh et al., 2004). As such, the increased relative abundance of these phyla is attributable to the corresponding improvement in soil chemical attributes with TSR on the ROWs. In addition, some agronomic practices can contribute to the improvement in the abundance of soil microorganisms. For example, Esmaeilzadeh-Salestani et al. (2021) showed a significant increase in the abundance of microbial communities after a five year-long crop rotation. Similarly, Qiu et al. (2020) showed that retaining straw on cropland significantly increased fungal biomass. The ROWs sampled in our study were constructed on cropland under crop rotations. Crops promote the proliferation of soil

microorganisms in the rhizosphere by producing exudates which provide carbon and energy to the microorganisms (Esmaeilzadeh-Salestani et al., 2021).

Conversely, the relative abundances of Bacteroidetes, Firmicutes, Ascomycota and Mucoromycota were greater on the 5-yr ROW than on the off-ROW on Site 2. These results corroborate those from other studies, which showed a greater abundance of Firmicutes on reclaimed landscapes disturbed by mining than on undisturbed sites (Li et al., 2016; Li et al., 2018). Firmicutes proliferate under poor nutrient and dry conditions (Li et al., 2016). The presence of the pipeline actively transporting pressurized fluids creates an environment of elevated soil temperature and reduced soil moisture on the ROWs (Naeth et al., 1993; Halmová & Fehér, 2014), which could have resulted in the observed trend in Firmicutes. However, our results are at variance with those from other studies, which showed less Bacteroidetes on undisturbed sites than on reclaimed sites (Li et al., 2016; Philippot et al., 2024). Ascomycota and Basidiomycota are the most abundant fungal phyla in the upper layers of the soil and are most effective in degrading crop residues due to their complimentary enzyme pools (Manici et al., 2024). The high relative abundances of Ascomycota and Basidiomycota on the 5-yr ROW than on the off-ROW reflects the SOM content of the treatments. Although the SOM content on the 5-yr ROW was statistically similar to the off-ROW, it could have been large enough to promote the multiplication of these fungal phyla.

4.5.4 Relationship between soil attributes and microbial community attributes

Pearson correlation analysis revealed significant relationships between some soil chemical properties and soil microbial community attributes. Correlations with soil chemical attributes are bi-directional since microorganisms not only respond to changes in soil chemical attributes but can also modify the soil environment (Philippot et al., 2024). Our results showed that Ascomycota,

Actinobacteria, and Firmicutes had significant and positive relationships with SOC, NO₃-N, Olsen-P, available K, Ca, and Mg. These results agree with those from other studies, which showed similar relationships between microbial attributes and soil chemical properties (e.g., Deng et al., 2018; Wang et al., 2024; Xiang et al., 2024). The results suggest that the recovery of soil microbial communities on reclaimed ROWs could be accelerated by the addition of SOM and fertilizer nutrients. Soil organic matter and available nutrients provide energy to soil microorganisms, thus promoting their proliferation. Several studies have also shown that the abundance of Actinobacteria (Qiu et al., 2020) and Ascomycota (Manici et al., 2024) increase with organic matter addition. However, these authors also reported an increase in Acidobacteria and Basidiomycota, which was in contrast with our results. Acidobacteria are acidophilic bacteria which are normally abundant in acidic conditions (Deng et al., 2018). Additionally, they also proliferate in fertilized soils because N fertilizers can slightly acidify the soil (Yang et al., 2021). As such, several studies showed a significant correlation between Acidobacteria with pH. However, in our study, the relative abundance of Acidobacteria was not correlated with soil pH. This could be due to the similarity in soil pH across treatments and sites. Similarly, the abundance of Proteobacteria has been shown to be correlated with soil C availability (Deng et al., 2018), which we did not detect in our study.

4.6 Conclusion

Our work is one of the few studies to investigate the impacts of underground pipeline construction on microbial communities on cropland using genomic techniques despite the fundamental roles played by soil microorganisms in soil health and productivity. Our findings showed that bacterial and fungal community diversity indices were lower on younger pipeline ROWs and improved with TSR to levels similar to those on the off-ROWs. Additionally, TSR

influenced the composition of bacterial and fungal communities at the phylum level. However, some bacterial and fungal phyla either did not respond to increasing TSR or responded in the opposite direction. This was not surprising considering that different bacteria and fungi require varying optimum conditions to grow and play various roles in the ecosystem. These results suggest that underground pipeline construction activities have deleterious effects on some bacterial and fungal phyla. However, the impacted microbial communities showed evidence of recovery over time. Nonetheless, the results confirmed our hypotheses and call for further studies to examine ways to accelerate the recovery of the soil microbiome on pipeline ROWs. Although our findings are just a snapshot of the ecological changes on the ROWs with increasing TSR, they show that microbial community attributes are impacted by pipeline construction and, therefore, should be considered in the assessment of the ecological recovery of underground pipeline ROWs on cropland. More comprehensive studies are recommended to investigate the underlying mechanisms driving these dynamics to enhance our understanding of the recovery of microbial communities on reclaimed ROWs.

4.7 References

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5. A BIOASSAY OF CROP RESPONSE TO FERTILIZER NUTRIENTS IN SOILS FROM A CHRONOSEQUENCE OF RECLAIMED NATURAL GAS PIPELINE RIGHTS-OF-WAY

5.1 Abstract

Construction of underground pipelines on cropland impacts the soil ecosystem, resulting in crop yield losses persisting for several years after right-of-way (ROW) reclamation. Information on whether higher than recommended fertilizer application rates could improve productivity of reclaimed ROWs is scarce. The objective of this growth room bioassay was to determine the productivity response of a chronosequence of reclaimed ROWs to differential fertilizer rates. Bulk soil samples were collected from the 0-20 cm layer on two ROWs of different ages (i.e., time elapsed since reclamation, TSR) and from undisturbed locations (off-ROW) on the same field. The experiment was laid out in a completely randomized design with a 3×3 factorial treatment structure and three replications per treatment. The factors were TSR (4 and 10 yr plus off-ROW) and fertilizer application rates (no fertilizer (Control); recommended rate (100%) of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S); and 150% of the recommended N, P, K, and S rates). The recommended nutrient rates produced 66% lower canola dry matter yield (DMY) and 45% lower wheat DMY in the 4-yr ROW soil relative to the off-ROW soil. Increasing the rate to 150% of the recommended rate significantly increased canola and wheat DMY in the 4-yr ROW soil to levels similar to those in the off-ROW soil receiving the recommended rate. Nitrogen uptake by canola and wheat was 30-45% lower, while P uptake was 40-55% lower in the ROW soils than in the off-ROW soil. These results indicate incomplete recovery of the ROW soils within at least the first 4 yr following completion of reclamation. However, crop yields were not significantly

different between the 10 yr ROW and the off-ROW at 100% fertilizer rate suggesting that productivity had been restored to off-ROW levels by year 10 following reclamation. Findings from this study will contribute towards the formulation of strategies for improving the productivity of reclaimed ROWs on cropland.

5.2 Introduction

Pipeline construction activities impact soil productivity through their negative effects on soil chemical, physical, and biological properties (Soon et al., 2000a; Shi et al., 2015). Lower crop yields on reclaimed pipeline rights-of-way (ROWs) than adjacent undisturbed sites (off-ROW) have been attributed to several factors, including increased soil penetration resistance and bulk density (Tekeste et al., 2019; Ebrahimi et al., 2022), reduced hydraulic conductivity (Soon et al., 2000a), and lower plant available nutrients (Soon et al., 2000a; Brehm et al., 2023). Some studies have shown that yield losses on reclaimed ROWs are long-lasting (e.g., Brehm et al., 2023; Muitire et al., 2024a). Persistent yield depressions on reclaimed ROWs could be due to the microenvironment created by the presence of the pipelines and their contents. The presence of pipelines actively transporting compressed fluids has been shown to elevate soil temperatures on ROWs, thereby significantly reducing soil moisture content (Naeth et al., 1993; Shi et al., 2015). Altering soil temperature and soil moisture regimes could also impact crop growth and development, particularly during dry seasons and in semiarid to arid regions where soil moisture is generally limiting (Naeth et al., 1993; Halmová et al., 2017).

Of all pipeline construction activities, soil excavation and salvaging, and trench backfilling are cited as the most deleterious activities to plant nutrient availability (Shi et al., 2015; Brehm and Culman, 2023). This is because these activities can lead to admixing of the relatively fertile topsoil and the less fertile subsoil. Lower plant available nutrient concentrations on reclaimed

ROWs relative to the off-ROWs are well-documented. For example, Shi et al. (2015) examined the impact of pipeline construction on crop productivity and soil properties in western China and reported lower concentrations of total nitrogen (N), available N, total phosphorus (P), available P, and soil organic matter (SOM) on ROWs than on off-ROWs 6 to 8 yr after reclamation. The authors also reported lower crop yields on the ROWs, which they attributed to reduced plant nutrient availability. In a quantitative synthesis of 34 studies from across the world on the impact of pipeline construction on soil and plant ecosystems, Brehm and Culman (2022) reported a 97.3% mean decrease in total N concentration across 11 studies, which they attributed to pipeline construction activities. However, the extent of pipeline construction impacts on soil ecosystems depends on several factors, including soil texture (Soon et al., 2000), season of pipeline construction (winter vs. summer) (Nielsen et al., 1990; Dessserud et al., 2010), and the installation method and equipment (Tekeste et al., 2019).

The application of fertilizers and manure, either singly or in combination, has been shown to improve the productivity of reclaimed soils to levels similar to those of adjacent undisturbed soils. However, most of these studies were conducted on non-cropland soils, such as grasslands and forested lands, disturbed by anthropogenic activities other than pipeline construction (e.g., Sloan and Jacobs, 2013; Buss et al., 2018). In these studies, fertilizer application enhanced vegetation establishment. Only a few studies have examined the effects of fertilizer application on reclaimed pipeline ROWs on cropland. For example, Nielsen et al. (1990) observed accelerated silking and an increase in corn yields in fertilizer and manure treatments compared to unfertilized plots on reclaimed pipeline ROWs on clay, loam, and loamy sand soils in Alberta, Canada. On grassland and forested land, fertilizers are usually applied during the early phases of the revegetation process to aid vegetation establishment (Guichon et al., 1983). The key question to be answered is whether

inferences from studies on grasslands and forested lands can be extended to reclaimed natural gas pipeline ROWs traversing Canadian prairie croplands.

Although studies on crop productivity on reclaimed ROWs generally agree on the underlying causes of reduced productivity on the ROWs, none have examined the changes in fertilizer nutrient use efficiency (NUE) indices. Our study is one of the few studies to investigate the influence of fertilizer rates on optimizing productivity on reclaimed ROWs on cropland in the Canadian Prairies. By examining nutrient efficiency indices on reclaimed ROWs and on off-ROWs, our study provides insights into the nutrient-related factors limiting crop yields on reclaimed ROWs. Various NUE indices with different applications are used in agricultural research (Congreves et al., 2021; Dobermann, 2007). Dobermann (2007) emphasizes the importance of utilizing multiple efficiency indices to fully understand the factors governing nutrient uptake and use efficiency in production systems. In addition, the choice of indices depends on the objectives of the study (Ernst et al., 2020). For instance, fertilizer based indices are applicable to situations where the contribution of applied fertilizer toward yield is to be explored while plant-based indices are useful for identifying plant genotypes with enhanced capability of allocating growth towards the economic components of plants (Congreves et al., 2021; Dobermann, 2007). In our study, fertilizer-based indices were employed to explore the impacts of ROW age on fertilizer nutrient recovery and utilization.

Results from our field study (Chapter 2) suggested that persisting yield losses on reclaimed ROWs relative to off-ROWs might be due to the impacts of pipeline construction activities and the presence of pipelines actively transporting compressed natural gas (Muitire et al., 2024a). The main objective of this growth room bioassay was to determine if fertilizer management could improve crop yields on reclaimed ROWs in the absence of active underground natural gas

pipelines. The specific objectives were to (1) determine biomass yield response to varying fertilizer application rates of spring wheat and canola grown in soils from ROWs of different ages and (2) determine nutrient uptake and N efficiency indices for canola and spring wheat as affected by fertilizer nutrient application rate and ROW age. We hypothesized that, in the absence of active pipeline effects, crop yields on reclaimed ROWs of different ages (i.e., time since reclamation, TSR) would be optimized by increasing the fertilizer application rate. Findings from this study will contribute towards a better understanding of the impacts of underground pipeline construction and operation on soil productivity and, ultimately, to the formulation of strategies for improving crop yields on underground natural gas pipeline ROWs.

5.3 Material and methods

5.3.1 Soil collection

Soils were collected from a field site in Farmington (55° 54' N; 120° 30' W), northeast British Columbia, Canada. The region is characterized by cold winters and short, warm summers. Long-term (1991-2020) seasonal mean temperatures range from -9.8 °C in the winter (November to February) to 13.2 °C in the growing season (May to September) (Environment and Climate Change Canada, 2024). Annual precipitation averages 463.6 mm, of which 292.3 is rainfall (Environment and Climate Change Canada, 2024). The dominant soils are acid to neutral Orthic Grey Luvisols (Boralfs in the U.S. Taxonomy) (B.C. Soil Information Finder Tool, 2018).

Bulk soil samples were collected after crop harvest in September 2021 from the 0-20 cm soil layer on two natural gas pipeline ROWs that had been reclaimed 4 and 10 yr prior (i.e., TSR of 4 and 10 yr) and from adjacent undisturbed transects (off-ROW) in the same field. The off-ROW soil samples were collected 50 m from the pipeline ROWs. To capture variability on the

ROWS and the off-ROW transects, bulk soil samples were collected from three locations (25 m apart) along each ROW and off-ROW. The soils were separately packed into 20-L pails, transported to the Land Remediation Lab, University of Manitoba, in Winnipeg, Canada, and stored in a cool area until processing. Prior to setting up the experiment, the bulk soil samples were air-dried and passed through a 4-mm sieve to remove small stones and plant residues, sub-sampled (100 g), and analyzed for particle size distribution, pH, and baseline concentrations of micro and macro plant nutrients.

5.3.2 Experimental design and setup

The experiment was laid out as a completely randomized design with a 3×3 factorial treatment structure replicated three times. The factors were TSR (4 yr, 10 yr, and off-ROW) and fertilizer nutrient (N, P_2O_5 , K_2O , and S) application rate (no fertilizer applied (control), recommended rate (100%), and $1.5 \times$ the recommended rate (150%). The 100% rate is the fertilizer rate that is typically recommended for wheat and canola in the region (B.C. Ministry of Agriculture, 2015). The fertilizers used to supply the above nutrients were urea, monoammonium phosphate (MAP), and potassium sulphate (K_2SO_4) (Table 5.1). Canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) were grown in pots in two concurrent sequences (canola-wheat-canola and wheat-canola-wheat) and three subsequent phases resulting in 54 experimental units in each phase.

Table 5.1 Recommended fertilizer application rates for wheat and canola.

Nutrient	Source	Formulation	Rate (g m ⁻²)†	
			Wheat	Canola
N	Urea	46-0-0-0	8.0	7.0
P	MAP	11-52-0-0	1.7	3.4
K	K ₂ SO ₄	0-0-50-17	1.1	3.4
S	K ₂ SO ₄	0-0-50-17	0.5	1.1

†Actual nutrient rate not adjusted for other sources

The experimental units were 12.5 L pots (23.5 cm L × 23.5 cm W × 18.5 cm H). The pots were filled with 7 kg air-dried and sieved soil packed to attain a bulk density of 1100 kg m⁻³. Each pot received a 20-mL aliquot of a full-strength micronutrient solution prepared according to Zvomuya et al. (2006) (Table 5.2). In crop phases 2 and 3, each pot received a 20-mL aliquot of the half-strength micronutrient solution. Reverse osmosis (RO) water was added to the potted soils to bring the water-filled pore space (WFPS) to 71% for the off-ROW soil, 60% for the 4-yr ROW soil, and 77% for the 10-yr ROW soil, corresponding to the container capacities of the respective soils, which closely approximate their field moisture capacities (Katanda et al., 2019). The pots were weighed and stored in the growth room for 24 h prior to planting. Subsequently, the pots were weighed every day and watered with RO water as needed to replace moisture lost via evapotranspiration.

Table 5.2 Full-strength solution added to all treatments before planting in crop Phase 1.

Element	Source	Rate (mg kg ⁻¹ soil)
Zn	ZnCl ₂	4.0†
Mn	MnCl ₂ .4H ₂ O	2.0
Mo	NaMoO ₄ .2H ₂ O	0.4
B	H ₃ BO ₃	1.0
Cu	CuSO ₄ .5H ₂ O	1.0
Fe	Fe.EDTA	4.0

†The amount of the actual element applied to the pot in phase 1. In phases 2 and 3, a 50% rate was used

One seed row measuring 2.5 cm wide and 2 cm deep was marked across the center of each pot. Sixteen canola seeds were planted 2.5 cm apart at the 2-cm depth while 40 spring wheat seeds were planted 1.2 cm apart at the 2-cm depth. Seven days after emergence, canola seedlings were thinned to 8 plants per pot to give a plant density of 145 plants m⁻² while wheat was thinned to 20 plants per pot for a plant density of 362 plants m⁻². All granular fertilizers were applied in a band 2.5 cm to the side of the seedrow and 2.5 cm below the seed (i.e., 4.5 cm below the soil surface). The same fertilizer application rates were used for the three crop phases. In crop phase one, N, P, K, and S were applied as granular fertilizers. However, in phases two and three, all fertilizers except MAP were applied in a solution to prevent the toxicity symptoms (yellowing, curling, and drying of leaves) observed in the ROW soils in phase one.

The growth room was maintained at 22 °C during the 16-h photoperiod and 16 °C during the dark hours, 65% relative humidity, and a light intensity of 270 μmol m⁻² s⁻¹ of photosynthetically active radiation. The plants were harvested at the early flowering stage (i.e., when plants in > 50% of the pots were at the early flowering stage) at approximately 42-49 d after emergence for above-ground biomass yield determination. Harvesting was accomplished by

cutting the plants approximately 2.5 cm above the soil surface using clippers. Harvested plant material was dried at 60 °C for 72 h and then weighed to determine dry matter yield (DMY). Dry tissue samples were ground to pass through a 2-mm sieve prior to laboratory analysis. Canola grain yield is highly and positively correlated with DMY (Zhang & Flottmann, 2016). Therefore, the DMY we obtained in this study reflects treatment effects on grain yield.

After harvest, the pots were emptied and soil from each pot was thoroughly mixed separately. Plant roots were retrieved from the soil, cut into < 2 cm pieces, and incorporated into the soil. The soil was re-potted and stored in the growth room until the start of the next phase. Planting, watering, fertilizer application, and harvesting for phases two and three proceeded as described for phase one with the stated modifications.

5.3.3 Laboratory analysis

Dry plant biomass from each pot was analyzed for nitrogen (N) and phosphorous (P) concentrations. Soil samples were analyzed for extractable macronutrients (NO₃-N, Olsen-P, calcium (Ca), magnesium (Mg), potassium (K), and sulfate sulfur (SO₄-S)), micronutrients (zinc, copper, iron, magnesium, and manganese), pH, electrical conductivity (EC), and soil organic carbon (SOC). Total carbon (TC) was determined using the dry combustion method (Nelson and Sommers, 1996), while inorganic carbon (IC) was estimated by pressure calcimetry after reacting the soil with 15% HCl (Loeppert and Suarez, 1996). Soil organic carbon (SOC) was calculated as TC minus IC (Nelson & Sommers, 1996). Electrical conductivity (EC) and soil pH were measured using an EC meter (Accumet® Basic, AB30) and a pH meter (Accumet® Basic, AB15), respectively, in a 1:2 soil to deionized water paste. Nitrate-N was measured colorimetrically following reduction with copperized cadmium (Gelderman & Beegle, 2015). Available P (Olsen P) was determined by the ascorbic acid-ammonium molybdate method (Murphy & Riley, 1962)

following extraction (1:10 soil to solution ratio) with 0.5 M sodium bicarbonate at a pH of 8.5 (Olsen et al., 1954). Sulfate S was extracted with 0.12 M KCl and determined by turbidimetry (Franzen, 2015). Concentrations of K, Ca, Mg, and micronutrients were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Warnacke & Brown, 2015). Cation exchange capacity (CEC) was obtained by summing the concentrations of K, Ca, Mg, and Na in ammonium acetate extracts (pH 7) (Peters et al., 2015).

5.3.4 Calculations

Nutrient uptake (Uptake) by the crops from each treatment in each crop phase was determined as:

$$\text{Uptake (mg kg}^{-1} \text{ soil)} = \text{DMY} \times \text{Nutr}_{\text{conc}} \quad [5.1]$$

where DMY is crop dry matter yield (g kg⁻¹ soil) and Nutr_{conc} is the tissue nutrient concentration (mg g⁻¹).

The following indices were calculated for N in both canola and wheat; N recovery efficiency (NRE, %) (Eq. [5.2]), agronomic efficiency (AE) (Eq. [5.3]), partial factor productivity (PFP) (Eq. [5.4]), and fertilizer contribution rate (FCR, %) (Eq. [5.5]). In these equations, *Uptake_f* is N uptake (g kg⁻¹ soil) in fertilized pots, *Uptake_c* is N uptake (g kg⁻¹ soil) in unfertilized check pots, *Yield_f* is DMY (g) in fertilized pots, and *Yield_c* is DMY (g) in unfertilized check pots. The difference method used for NRE, FCR, and AE assumes that the uptake of nonfertilizer nutrients from the soil is the same for fertilized and nonfertilized plants (Zvomuya et al., 2003).

$$\text{NRE} = \frac{\text{Uptake}_f - \text{Uptake}_c}{\text{N applied (g)}} \times 100 \quad [5.2]$$

$$\text{AE} = \frac{\text{Yield}_f - \text{Yield}_c}{\text{N applied (g)}} \quad [5.3]$$

$$PFP = \frac{Yield(g)}{N\ applied(g)} \quad [5.4]$$

$$FCR = \frac{Yield_f - Yield_c}{Yield_f} \times 100 \quad [5.5]$$

5.3.5 Statistical analysis

Analysis of variance was conducted using the Generalized Linear Mixed Model procedure (PROC GLIMMIX) in SAS[®] OnDemand for Academics version 9.04.01 (SAS Institute Inc, Cary, NC) to determine the effect of crop phase, TSR, fertilizer rate, and their interactions on DMY, nutrient uptake, and N indices. Crop phase, TSR, and fertilizer rate were modelled as fixed effects. The MEANS procedure (SAS Institute Inc, Cary, NC) was used to calculate treatment means and standard deviations for soil baseline properties. The UNIVARIATE procedure (SAS Institute Inc, Cary, NC) indicated that DMY, AE, PFP, nutrient concentration, and nutrient uptake data were normally distributed according to the Shapiro-Wilk statistic (W , $\alpha = 0.05$). NRE and FCR data followed a beta distribution, which was specified using the DIST function in the MODEL statement of PROC GLIMMIX. DMY in crop phase one was lower in the fertilized pots in the 10-yr ROW and some replicates of the 4-yr ROW, likely due to fertilizer toxicity on the seedlings. Due to this, the difference method used to calculate N indices yielded negative values in some replicates. Therefore, phase one data were not included in calculating N efficiency indices. Treatment means were separated using Tukey's multiple comparison procedure at $\alpha = 0.05$.

5.4 Results

5.4.1 Baseline soil properties

Prior to the start of the bioassay, soil samples from the ROW and off-ROW were analyzed for selected soil properties (Table 5.3). The concentration of SOC in the off-ROW soil was 4 and 2 times greater than in the 4- and 10-yr ROW soils, respectively. Soil pH of the 4- and 10-yr ROWs was acidic while it was slightly alkaline for the off-ROW. Cation exchange capacity was lowest in the 4-yr ROW soil than in the 10-yr ROW and the off-ROW soils. The concentrations of NO₃-N, Olsen-P, extractable K and all micronutrients were greater in the off-ROW soil than in the 4- and 10-yr ROW soils except for iron, which was lowest in the off-ROW soil. Differences in macro and micro-nutrients between the 4- and 10-yr ROWs were small.

Table 5.3 Selected soil baseline properties.

Analyte	4-yr ROW	10-yr ROW	Off-ROW
SOC (g kg ⁻¹)†	24±0.1‡	47±0.8	102±0.7
Nitrate-N (mg kg ⁻¹)	87.5±24.7	53.0±11.3	113±81.3
Olsen P (mg kg ⁻¹)	38±5.7	56±1.4	230±19.1
K (mg kg ⁻¹)§	150±14.8	157±2.8	542±26.2
SO ₄ -S (mg kg ⁻¹)	60	44±11.3	60
Ca (mg kg ⁻¹)	1690±69.3	893±18.4	4166±137.9
Mg (mg kg ⁻¹)	271±13.4	118±4.2	681±40.3
Na (mg kg ⁻¹)	35±8.5	18±4.2	43.5±4.9
Zn (mg kg ⁻¹)	3.5±1.5	3.0	13.4±4.9
Fe (mg kg ⁻¹)	123±50.3	128	108±36.9
Mn (mg kg ⁻¹)	26.3±23.4	63.6	10.3±2
Cu (mg kg ⁻¹)	1.2±0.2	1.4	2.3±0.7
B (mg kg ⁻¹)	0.7±0.6	0.2	3.9±0.9
pH	5.9±0.1	4.9±0.2	7.3±0.1
CEC (cmol _c kg ⁻¹)	13.6±2.8	25.9±28.1	28.2±1.1
Particle size distribution (%)			
Sand	29	39	39
Silt	46	44	40
Clay	25	17	21
Texture	Loam	Loam	Loam

†SOC, soil organic carbon; P, Olsen-P; K, potassium; SO₄-S, sulfate-sulfur; Ca, calcium; Mg, magnesium; Na, sodium; Zn, zinc; Fe, iron; Mn, manganese; Cu, copper; B, boron; CEC, cation exchange capacity

‡Mean ± standard deviation. There was one analytical sample replicate for micronutrients on the 10-yr ROW and for particle size distribution in the ROWs and off-ROW. Standard deviation for SO₄-S was zero in the 4-yr ROW and the off-ROW

§Ammonium acetate (pH 7.0) extractable K, Ca, Mg; DTPA-extractable Zn, Fe, Mn, Cu

5.4.2 Dry matter yield

5.4.2.1 Canola

The effect of TSR on canola DMY varied with fertilizer rate and crop phase, as indicated by the significant ($p = 0.001$) $\text{TSR} \times \text{rate} \times \text{phase}$ interaction (Table 5.4). In the control, DMY was significantly lower for the 4-yr ROW soil than the off-ROW soil in phases one (by 63%) and three (by 84%), but the TSR effect was not significant in phase two (Figure 5.1a). At the recommended fertilizer rate (100%), canola DMY in phase one in the 4-yr ROW soil was 66% lower than in the 10-yr ROW soil and 53% lower than in the off-ROW soil, while the DMY was 28% lower in the 10-yr ROW soil than in the off-ROW soil (Figure 5.1b). Increasing the fertilizer rate to 150% of the recommended rate increased canola DMY in the 4-yr ROW soil by 124%. Thus, at the 150% fertilizer rate, canola DMYs in the 4- and 10-yr ROWs were similar to the DMY in the off-ROW in all three crop phases except for the low DMY observed in the 10-yr ROW in phase one, which, as explained above, could have been due to fertilizer toxicity (Figure 5.1c).

Table 5.4 Canola and wheat dry matter yield (DMY) and N and P uptake as affected by crop phase, time since reclamation, and fertilizer application rate.

Effect	Biomass		Uptake			
	Canola	Wheat	Canola		Wheat	
	— g kg ⁻¹ soil —		N†	P	N	P
			mg kg ⁻¹ soil			
Phase						
1	6.8 b‡	7.7 a	183 b	24.3 a	233 a	23.5 a
2	7.4 a	6.0 b	212 a	21.6 b	97.0 b	16.0 b
3	3.4 c	5.4 b	52.0 c	11.8 c	74.7 b	12.1 c
TSR (yr)						
4	4.5 c	4.9 c	121 c	11.5 c	103 b	10.5 c
10	5.6 b	6.3 b	138 b	16.1 c	121 b	15.2 b
Off-ROW	7.7 a	8.0 a	189 a	29.7 a	181 a	25.8 a
Rate						
Control	4.4 b	4.9 c	84.4 b	14.6 b	94.3 c	14.2 b
100%	6.4 a	6.6 a	175 a	21.6 a	132 b	17.8 a
150%	6.8 a	7.6 a	188 a	21.1 a	179 a	19.6 a
			<i>P-value</i>			
Phase (P)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TSR	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rate (R)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TSR × R	0.0002	0.01	0.003	< 0.0001	0.04	0.01
TSR × P	0.001	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001
R × P	0.0004	0.01	< 0.0001	0.003	0.9	0.04
TSR × R × P	0.001	0.06	0.001	< 0.0001	0.1	0.6

†N, total nitrogen; P, total phosphorus; TSR, time since reclamation (yr)

‡Means followed by the same letter in a column under each effect are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

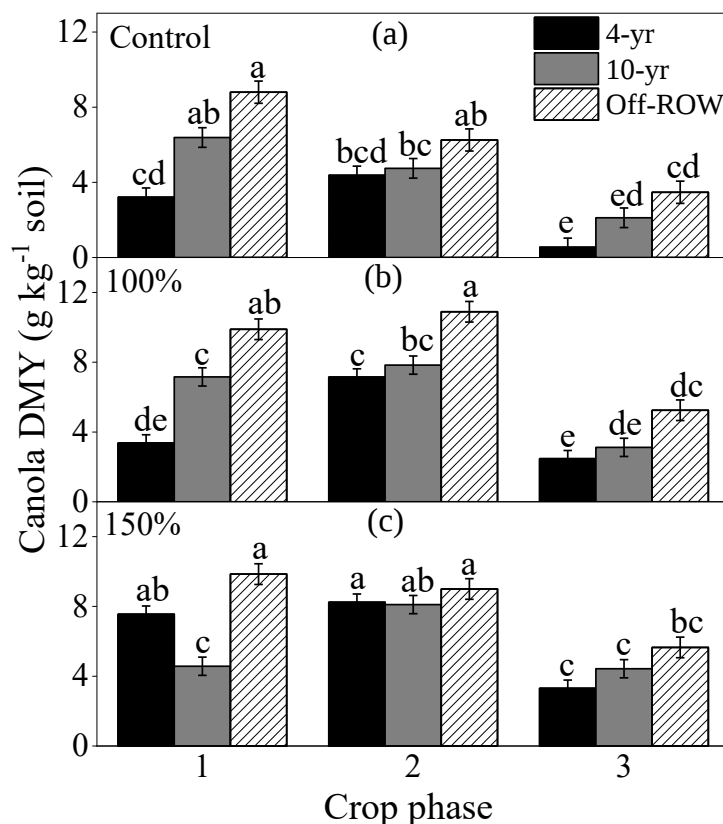


Figure 5.1 Canola dry matter yield (DMY) as affected by fertilizer application rate ((a) control; (b) 100%; (c) 150%), time since reclamation, and crop phase. Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.2.2 Wheat

Averaged across crop phases, the effect of TSR on wheat DMY varied with fertilizer application rate, as indicated by the significant ($p = 0.01$) TSR $\times$ rate interaction (Table 5.4). In the control, wheat DMY was 52% lower in the 4-yr ROW soil than in the off-ROW soil while DMY in the 10-yr ROW soil was not significantly different from that in the off-ROW soil (Figure 5.2). At the 100% rate, wheat DMY was 45% lower in the 4-yr ROW soil than in the off-ROW soil but did not differ significantly between the 10-yr ROW and the off-ROW. However, when the

fertilizer application rate was increased to 150%, wheat DMY did not differ significantly between ROW soils and off-ROW soils.

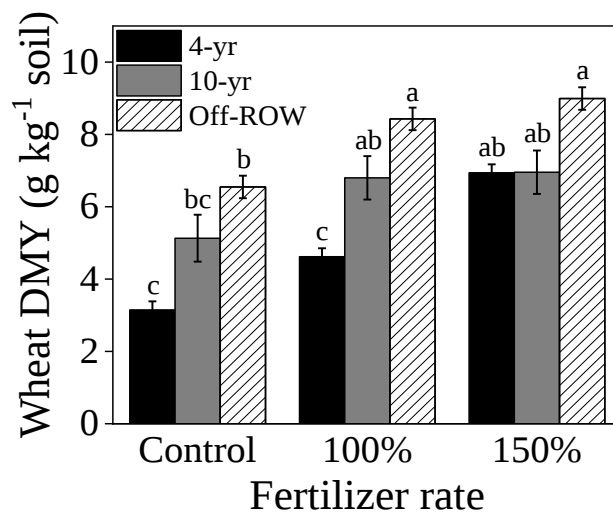


Figure 5.2 Wheat dry matter yield (DMY) as affected by TSR and fertilizer application rate. Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.3 Nitrogen and phosphorus uptake

5.4.3.1 Canola

The effect of TSR on canola N uptake varied with fertilizer application rate and crop phase, as indicated by the significant ($p = 0.001$) TSR $\times$ rate $\times$ phase interaction (Table 5.4). In the control, N uptake was 245% greater in the off-ROW soil than in the 4-yr ROW soil in crop phase one but the TSR effect was not significant in phases two and three (Figure 5.3a). At the 100% fertilizer rate, canola N uptake in the 4-yr ROW soil was 250% lower in phase one and 150% lower in phase two relative to the off-ROW soil whereas the TSR effect was not significant in phase three. However, at the 150% N rate, the TSR effect on canola N uptake was not significant in all crop

phases except for a significant decrease in N uptake in the 10-yr ROW relative to the off-ROW soil in phase one (Figure 5.3a).

There was also a significant ($p = 0.0001$) TSR $\times$ rate $\times$ crop phase interaction for P uptake by canola (Table 5.4). In the control, P uptake was 79% lower in the 4-yr ROW and 32% lower in the 10-yr ROW relative to the off-ROW soil in phase one (Figure 5.3b). At the 100% fertilizer application rate, P uptake in the off-ROW soil was 200% to 250% greater than in the ROW soils in phases one and two. Increasing fertilizer application rate to 150% did not improve canola P uptake in the 4- and 10-yr ROW soils to levels similar to the off-ROW in all crop phases (Figure 5.3b).

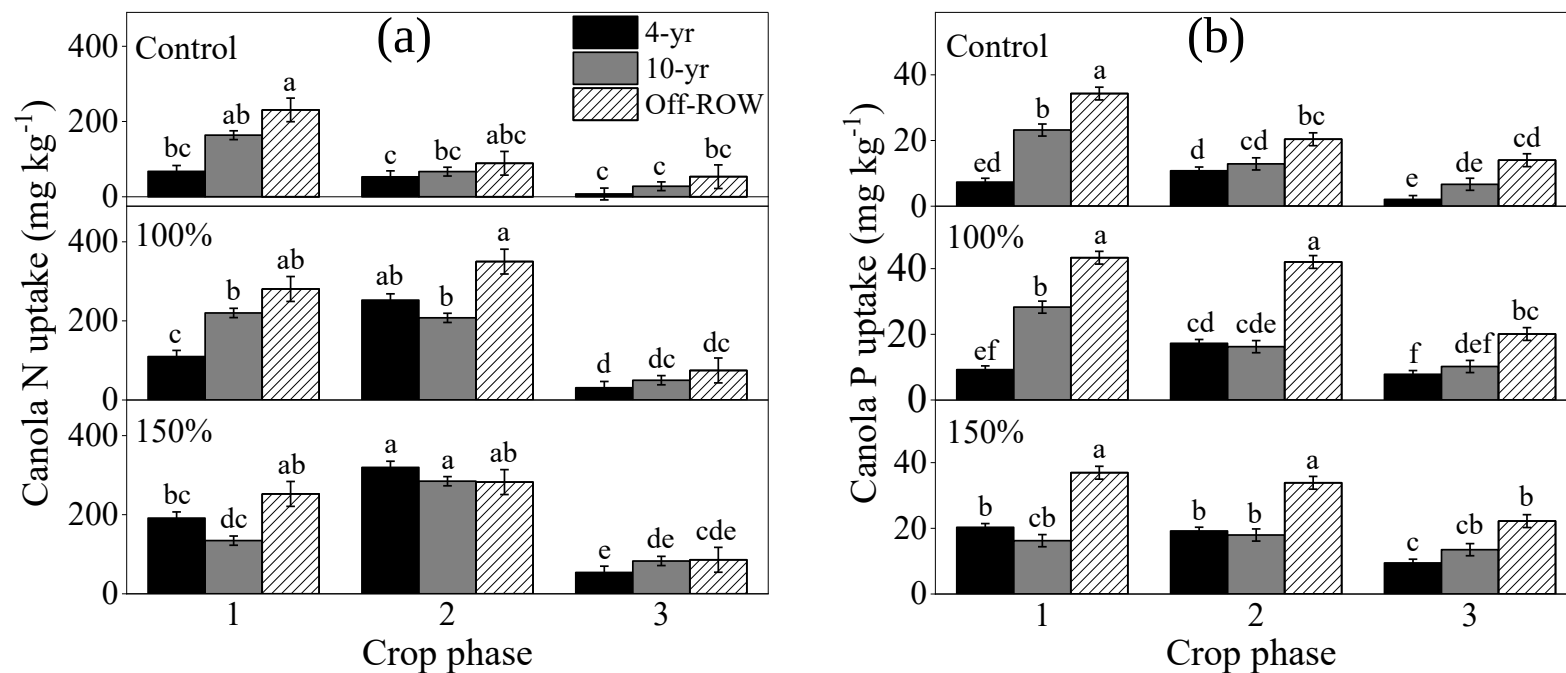


Figure 5.3 Effect of time since reclamation, crop phase, and fertilizer application rate on canola N (a) and P (b) uptake. Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.3.2 Wheat

Averaged across crop phases, there was a significant ($p = 0.04$) TSR $\times$ rate interaction for wheat N uptake (Table 5.4). In the control, N uptake was 250% greater in the off-ROW soil than in the 4-yr ROW soil, whereas N uptake did not differ significantly between the two ROWs (Figure 5.4a). At the 100% fertilizer rate, N uptake in the off-ROW soil was 125% greater than that in the 4-yr ROW soil and 61% greater than that in the 10-yr ROW soil. Increasing the fertilizer rate to 150% improved N uptake in the 4-yr ROW soil by 100% such that it was not significantly different from N uptake in the off-ROW soil. However, at the 150% fertilizer rate, N uptake in the 10-yr ROW soil was still significantly lower than in the off-ROW soil.

A significant ($p = 0.01$) TSR $\times$ rate interaction was also detected for P uptake (Table 5.4). Averaged across crop phases, P uptake in the off-ROW soil was 58% to 200% greater than in the 4- and 10-yr ROW soils except in the control where P uptake in the 10-yr ROW soil did not differ significantly from that in the off-ROW soil (Figure 5.4b).

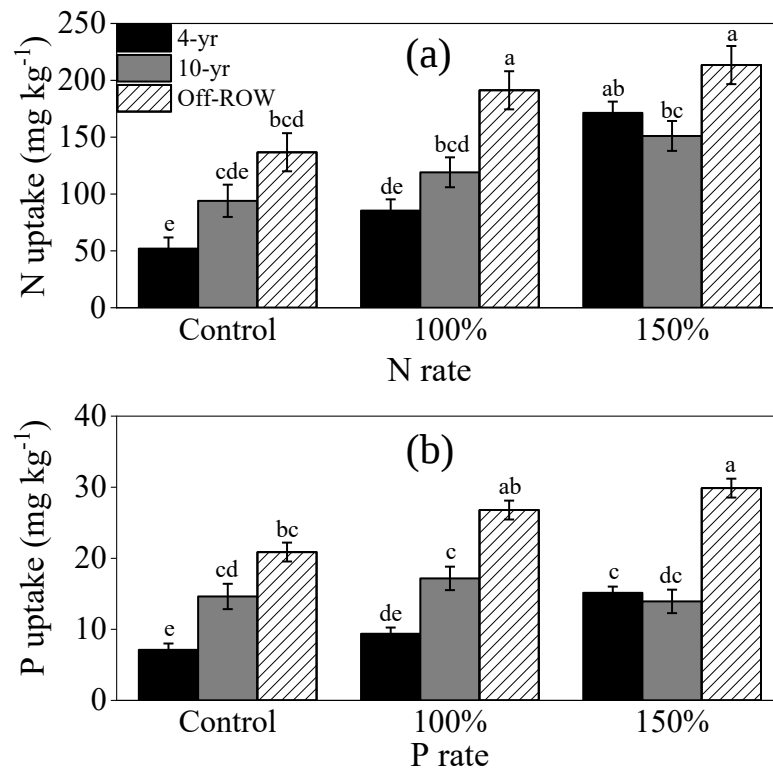


Figure 5.4 Effect of time since reclamation (TSR) and fertilizer application rate on wheat N (a) and P (b) uptake. Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

Table 5.5 Canola and wheat nutrient use efficiency indices as affected by crop phase, TSR, and fertilizer rate.

Effect	ARE [†]		AE		PFP		FCR	
	Canola	Wheat	Canola	Wheat	Canola	Wheat	Canola	Wheat
	%		g DM g ⁻¹				%	
Phase								
1	- [‡]	-	-	-	-	-	-	-
2	46.9 a§	40.2 a	28.1 a	16.3 b	70.2 a	44.0 a	39.2 b	36.9 b
3	25.2 b	34.0 a	15.6 b	21.6 a	32.4 b	42.0 a	52.8 a	53.3 a
TSR (yr)								
4	32.7 a	41.9 a	22.3 a	21.8 a	42.6 b	37.6 b	62.6 a	58.8 a
10	43.0 a	33.5 a	19.4 a	19.6 b	47.5 b	43.0 ab	36.5 b	45.0 b
Off-ROW	30.6 a	36.0 a	23.9 a	15.5 b	63.8 a	48.5 a	35.9 b	31.8 c
Rate								
Control	-	-	-	-	-	-	-	-
100%	34.0 a	41.4 a	24.9 a	19.9 a	60.3 a	48.8 a	43.0 a	41.3 b
150%	36.0 a	32.5 b	18.7 b	18.0 a	42.2 b	37.2 b	48.9 a	48.6 a
	<i>P-value</i>							
Phase (P)	< 0.0001	0.11	< 0.0001	0.01	< 0.0001	0.29	0.001	< 0.0001
TSR	0.12	0.23	0.30	0.01	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Rate (R)	0.63	0.02	0.01	0.32	< 0.0001	< 0.0001	0.08	0.01
TSR × R	0.02	0.50	0.01	0.73	0.001	0.04	0.05	0.66
TSR × P	0.29	0.48	0.28	0.26	0.79	0.40	< 0.0001	0.001
Rate × P	0.18	0.95	0.01	0.37	< 0.0001	0.92	0.06	0.38
TSR × P × R	0.08	0.21	0.09	0.51	0.16	0.39	0.21	0.64

[†]ARE, apparent nutrient recovery efficiency; AE, agronomic efficiency; PFP, partial factor productivity; FCR, fertilizer contribution rate; TSR, time since reclamation (yr)

[‡](-), Phase one data were not included in the analysis due to fertilizer toxicity. No values for the control since the difference method was used

§Means followed by the same letter in a column under each effect are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

5.4.4 Nitrogen efficiency indices

5.4.4.1 Nitrogen recovery efficiency

Across crop phases, the effect of TSR on canola NRE varied with nutrient application rate, as indicated by the significant ($p = 0.02$) TSR $\times$ rate interaction (Table 5.5). Nitrogen recovery efficiency under canola at the 100% N rate was 46% greater in the off-ROW soil than in the 4-yr ROW soil. At the 150% N rate, NRE in the off-ROW soil was 40% lower than that in the 4-yr ROW soil and 49% lower than that in the 10-yr ROW soils (Figure 5.5a). For wheat, NRE varied with fertilizer rate only ($p = 0.02$) (Table 5.5). Across crop phases and TSR, NRE decreased by 21% when the N rate was increased from 100% to 150%.

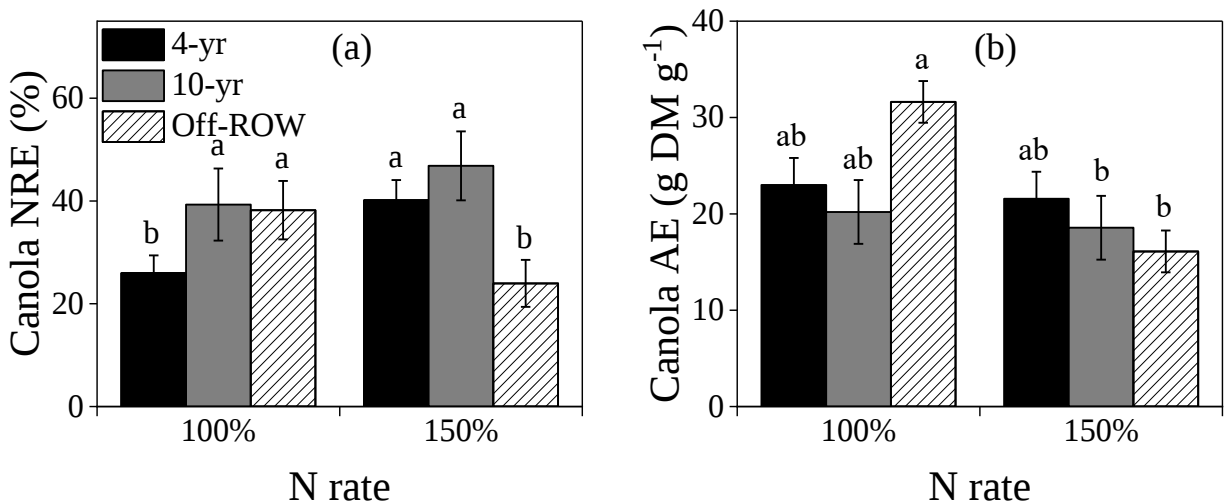


Figure 5.5 Effects of time since reclamation and N application rate on canola N recovery efficiency (NRE) (a) and agronomic efficiency (AE) (b). Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.4.2 Agronomic efficiency

The effect of TSR on AE under canola varied with fertilizer rate, as shown by the significant ($p = 0.01$) TSR $\times$ rate interaction (Table 5.5). Averaged across canola phases, AE was

39% greater in the off-ROW soil than in the 10-yr ROW soil at the 100% fertilizer rate, whereas the TSR effect was not significant at the 150% fertilizer rate (Figure 5.5b). Averaged across TSR, the effect of fertilizer rate on canola AE varied significantly ($p = 0.01$) with crop phase (Table 5.5). In phase two, canola AE was 59% greater for the 100% fertilizer rate than the 150% fertilizer rate. In phase three, AE did not differ significantly between fertilizer rates (Figure 5.6a).

There were significant crop phase ($p = 0.01$) and TSR ($p = 0.01$) effects on AE under wheat (Table 5.5). Agronomic efficiency was 24% greater in phase three than in phase two, while wheat AE in the 4-yr ROW soil was 11% greater than that in the 10-yr ROW soil and 41% greater than that in the off-ROW soil.

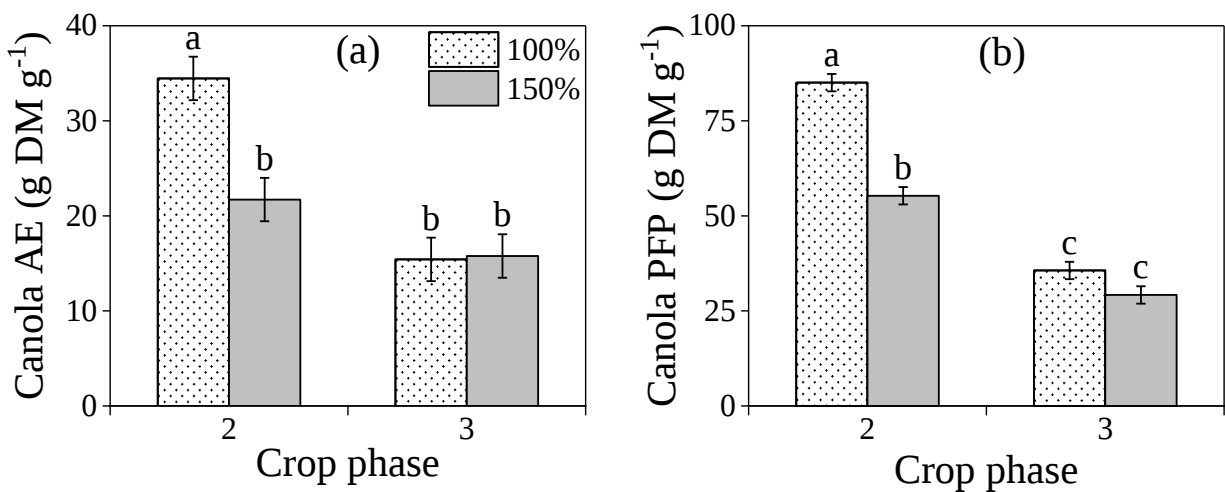


Figure 5.6 Effects of fertilizer application rate and crop phase on agronomic efficiency (AE) under canola (a) and partial factor productivity (PFP) (b). Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.4.3 Partial factor productivity

There was a significant ($p = 0.0001$) fertilizer rate $\times$ crop phase interaction for PFP under canola (Table 5.5). In canola phase two, PFP was 54% greater at the 100% fertilizer rate than at

the 150% rate but did not differ significantly between the fertilizer rates in phase three (Figure 5.6b). The effect of TSR on canola PFP also varied with fertilizer rate, as indicated by the significant ($p = 0.001$) TSR $\times$ rate interaction (Table 5.5). At the lower fertilizer rate, PFP in the off-ROW soil was 68% greater than in the 4-yr ROW soil and 47 % greater than in the 10-yr ROW soil, while no significant differences were detected for canola PFP at the 150% N rate (Figure 5.7a). There was also a significant ($p = 0.04$) TSR $\times$ rate interaction for PFP under wheat (Table 5.5). Significant differences in wheat PFP were detected at the 100% fertilizer rate, where wheat PFP in the 4-yr ROW soil was 28% lower than in the off-ROW soil, with no significant difference at the 150% fertilizer rate (Figure 5.7b).

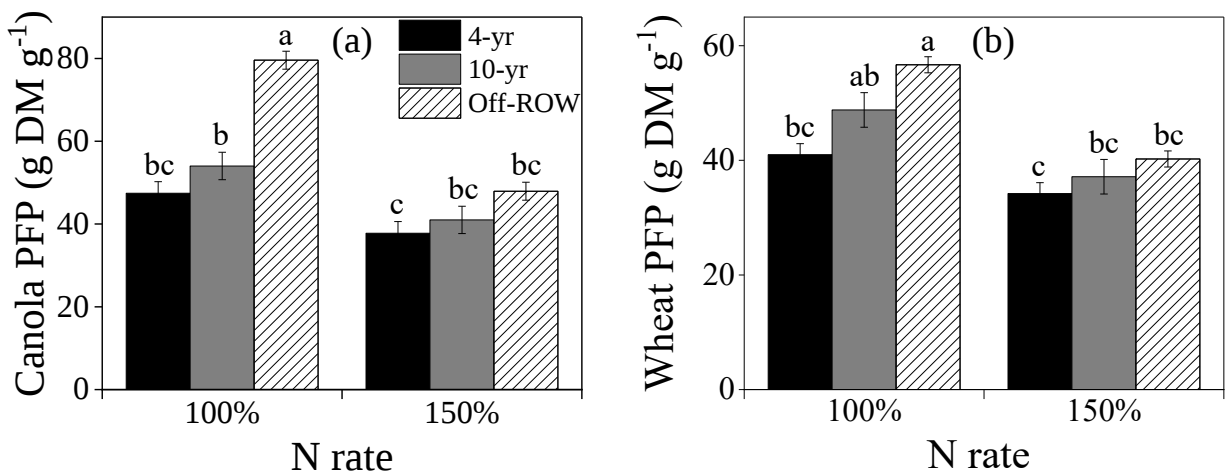


Figure 5.7 Time since reclamation and fertilizer application rate effects on partial factor productivity (PFP) under canola (a) and wheat (b). Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.4.4.4 Fertilizer contribution rate

Averaged across fertilizer rates, the effect of TSR on FCR varied with canola phase ($p = 0.0001$ for the TSR $\times$ crop phase interaction; Table 5.5). The TSR effect was not significant in phase two, while in phase three, FCR under canola was 105% greater in the 4-yr ROW soil than

in the 10-yr ROW soil and the off-ROW soil (Figure 5.8a). The TSR $\times$ crop phase interaction was also significant ($p = 0.001$) for FCR under wheat (Table 5.5). In wheat phase two, FCR was 36% lower in the off-ROW soil than in the 4-yr ROW soil, while FCR in the 10-yr ROW soil did not differ significantly from the off-ROW (Figure 5.8b). In phase three, wheat FCR in the off-ROW soil was 52% lower than in the 4-yr ROW soil and 41% lower than in the 10-yr ROW soil.

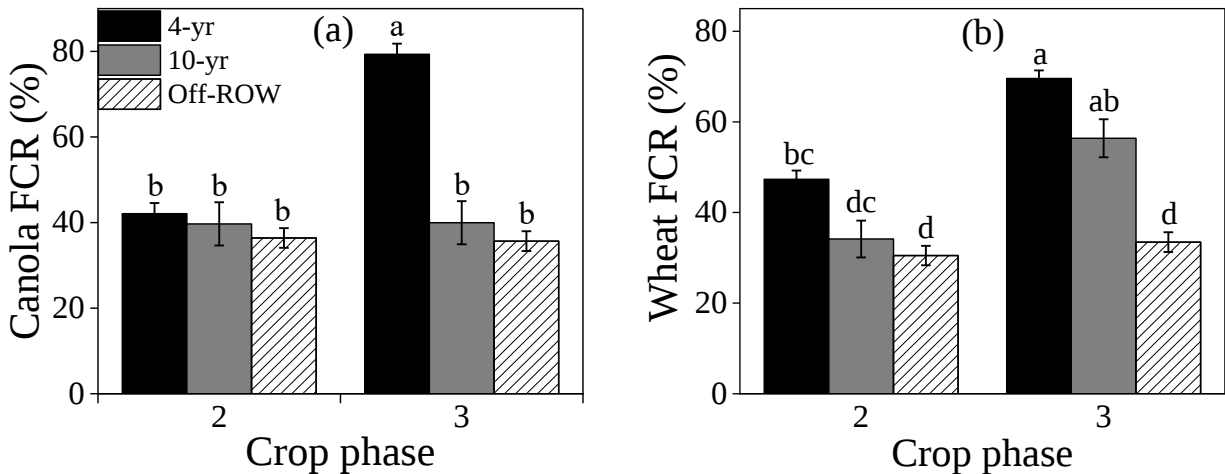


Figure 5.8 Effects of time since reclamation and crop phase on fertilizer contribution rate (FCR) under canola (a) and wheat (b). Bars with the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$). Error bars represent standard errors of the mean.

5.5 Discussion

5.5.1 Baseline properties

The off-ROW soils had greater concentrations of SOC, $\text{NO}_3\text{-N}$, Olsen-P, K, and all micronutrients, except iron, than the soils from reclaimed 4- and 10-yr ROWs. Soil pH was slightly alkaline for the off-ROW, while it was acidic for the ROWs. Low soil pH can reduce the plant-availability of nutrients such as P (Grant and Flaten, 2019). The observed differences in soil baseline properties could be reflective of the impacts of pipeline construction on the soil

ecosystem. These findings are consistent with results from several other studies, which showed lower SOC and plant available nutrient concentrations on reclaimed ROWs than on the off-ROW (e.g., Soon et al., 2000a; Shi et al., 2015; Brehm et al., 2015). Lower SOC on reclaimed ROWs has been attributed to the dilution of topsoil with subsoil (admixture), increased C mineralization, and reduced organic C inputs (Whitson, 2023). As shown in our and other studies, plant growth is typically lower on reclaimed ROWs than on off-ROWs. As a result, there is a slower buildup of soil organic matter, hence SOC pools, on reclaimed ROWs. As with SOC, lower plant-available nutrients on reclaimed ROWs have also been linked to the dilution of topsoil with subsoil (Soon et al., 2000a; Brehm & Culman, 2023). Lower plant-available nutrients on reclaimed ROWs could limit plant growth. As a result, the restoration of SOC on reclaimed ROWs could take many years (Naeth et al., 2020).

5.5.2 Crop biomass yield

We observed yellowing, curling, and dying of leaves on both canola and wheat seedlings in the fertilized soil from the 10-yr ROW and some replicates of the 4-yr ROW soil during the first two weeks following crop emergence in phase one of the experiment. These are typical fertilizer toxicity symptoms in canola and wheat seedlings (Pan et al., 2016). Canola seedlings are highly susceptible to N toxicity, particularly if the N fertilizer is placed in the seedrow (Johnston et al., 2002). Although all fertilizers were side-banded in phase one, the observed symptoms were consistent with fertilizer toxicity in canola. The symptoms, however, started to gradually disappear in the third week following crop emergence. Due to the fertilizer toxicity, crop DMY in some of the fertilized ROW soil pots were lower than DMY in unfertilized (control) pots. To prevent fertilizer toxicity in subsequent phases, all fertilizers were applied in solution in phases two and three, except for MAP, which was side-banded as granules.

At the recommended (100%) fertilizer application rate, DMY of both canola and wheat were lower in the 4-yr ROW than in the off-ROW soil. However, the 100% rate seemed sufficient to optimize crop yields in the 10-yr ROW soil, which produced similar DMY to the off-ROW soil. The recommended fertilizer rate applied in this study was likely suboptimal for the 4-yr ROW soil, which, although it had a higher $\text{NO}_3\text{-N}$ concentration (88 mg kg^{-1}) than the 10-yr ROW (53 mg kg^{-1}), had lower canola and wheat DMY than the 10-yr ROW. Lower crop productivity relative to off-ROWs is common on reclaimed underground pipeline ROWs and can persist for several years post-reclamation (e.g., Shi et al., 2015; Brehm et al., 2023; Muitire et al., 2024a). For example, reduced crop yields on cropland have been observed 3–8 yr after reclamation of ROWs (e.g., Soon et al., 2000; Brehm & Culman, 2023), although the yields gradually improved with increasing TSR. The low productivity on reclaimed ROWs has been mostly attributed to low plant nutrient availability in the affected soils.

Crop DMYs did not differ significantly between the 10-yr ROW soil and the off-ROW soil at the 100% fertilizer rate, indicating a significant improvement in ROW soil productivity as TSR increased from 4 to 10 yr. These results are in contrast with those from a field study conducted on the same reclaimed ROWs, which showed that, at the recommended fertilizer rate, crop yield reductions on reclaimed ROWs persisted beyond 12 yr following reclamation (Muitire et al., 2024a). The persistent yield losses were attributed to factors associated with the presence of operational natural gas pipelines, such as elevated soil temperature and reduced soil moisture on the ROWs relative to the off-ROW. This is particularly pertinent in the semi-arid study region, which receives less than 300 mm of rainfall during the growing season. Therefore, the disparity between results from the field study and this growth room study may reflect the impacts of active pipelines on the soil ecosystem. The increase in DMY as TSR increased from 4 to 10 yr could be

due to the observed improvement in soil properties, particularly SOC, which was two times greater in the 10-yr ROW than in the 4-yr ROW.

Our study is one of the few that explored the possibility of improving the productivity of reclaimed ROWs on cropland by increasing fertilizer application rates. Increasing the recommended fertilizer application rate by 50% improved canola and wheat DMY in the 4-yr ROW soil to a level similar to that in the off-ROW soil, suggesting that crop yields on the 4-yr ROW could possibly be optimized by increasing fertilizer application rates. Similar results have previously been reported in field studies where crop yields on reclaimed ROWs showed a positive response to increased fertilizer application rates. For example, in eastern Ontario and Quebec, Canada, corn height and dry matter yields on reclaimed ROWs became similar to those on the off-ROWs when N application rate was increased from 112 to 224 kg ha⁻¹ in Solonetzic and Chernozemic soils (Nielsen et al., 1990). Although this was a 100% rate increase, unlike in our study, where the rate was increased by 50%, crop yield generally improve with increasing fertilizer rate due to greater nutrient availability at the higher rates (Zhang & Flottmann, 2016; Zuo et al., 2020). However, with increasing fertilizer rates, crop yields will reach a peak beyond which a further increase in fertilizer rate will result in a diminishing return on crop yield (Zuo et al., 2020). This could have happened in the 10-yr ROW and off-ROW soils where the response to higher fertilizer rate was not significant. It is noteworthy that improved fertility status of the soil does not always translate to improved yields on reclaimed ROWs since disruption of soil physical and biological characteristics can also limit crop growth on reclaimed ROWs (Nielsen et al., 1990). These factors point to the need for further studies to ascertain the benefits of applying greater fertilizer rates to improve productivity on reclaimed ROWs.

5.5.3 Nitrogen and phosphorus uptake and use efficiency indices

At the 100% fertilizer rate, N and P uptake were lower in the 4-yr ROW soil than in the off-ROW soil for both wheat and canola. Increasing the fertilizer rate to 150% of the recommended rate increased N uptake in the 4-yr ROW soil to a level similar to that in the off-ROW soil for both crops. This trend was similar to that observed for DMY. The improvement in N uptake observed in the 4-yr ROW suggests a greater N availability at the higher fertilizer rate. However, P uptake did not respond to the higher fertilizer rate regardless of crop species, suggesting that the DMY gain observed in the ROW soils at the higher fertilizer rate was likely due to increased N availability, with little to no contribution from increased P supply. Our results on P uptake contradict those from other studies. For instance, Katanda et al. (2016) observed an increase in canola and wheat P uptake when the application rate was increased by 200% from 1.1 g P m⁻² to 2.2 g P m⁻² in a clay loam and a sandy soil. This is despite the fact that our recommended P rates were greater (1.7 g m⁻² for wheat and 3.4 g m⁻² for canola) than that used by Katanda et al. (2016). The lack of response to higher P rate in our study could be due to the variability in response among crop cultivars since not all wheat cultivars respond to P application in most soils (Yaseen & Malhi, 2009). Although there was a difference in nutrient uptake between the ROW soils and the off-ROW soils, tissue P concentrations in our study were within the critical ranges at flowering of 2.5 to 5 g P kg⁻¹ for canola (Grant & Bailey, 1993) and 1.3 to 3.9 g P kg⁻¹ for wheat (Rashid et al., 2005) (Table S5.1), indicating that there was no P deficiency in all soils. Tissue N concentrations in the unfertilized soils were in the deficiency range (< 20 g N kg⁻¹) for canola at the flowering stage (Grant & Bailey, 1993), while wheat tissue N concentrations were above the critical value of 38.9 g N kg⁻¹ DM for spring wheat at flowering in the Canadian prairies (Ziadi et al., 2010).

The effects of TSR and fertilizer application rate on crop productivity could be further explained by nutrient efficiency indices. This study explored fertilizer-based nutrient efficiency indices which indicate the contribution of fertilizer-applied nutrients toward crop yield (Dobermann, 2007; Congreves et al., 2021). Due to limitations associated with using individual indices, we used multiple indices in this study, to get a holistic insight into plant nutrient use dynamics (Van Eerd, 2007; Fixen et al., 2015). As previously explained, DMY was lower in fertilized pots in phase one due to suspected fertilizer toxicity, resulting in negative values of some indices in phase one. Therefore, phase one data were not included in the analysis. At the 100% fertilizer application rate, ARE, AE, and PFP for N in the off-ROW soil were mostly greater than in the 4-yr ROW soil and in some instances greater than in the 10-yr ROW soil as well. This trend uncovered the fundamental processes underlying the DMY discrepancies between the ROW soils and the off-ROW soils. Although there was no evidence of nutrient deficiency in the soils, these results suggest that more fertilizer nutrients were available for plant uptake in the off-ROW soil than in the ROW soils. Unlike the other indices, FCR indirectly indicates the contribution of inherent soil nutrients to crop yield. The higher FCR in the 4-yr ROW soil than in the 10-yr ROW and the off-ROW soils suggests that much of the observed yield gain observed in the 4-yr ROW soil was largely due to applied fertilizer nutrients than to inherent soil nutrients, indicating the relatively lower fertility of the 4-yr ROW soil than the off-ROW soil.

5.6 Conclusion

This is one of a few studies that evaluated the effect of varying fertilizer application rates on the productivity of reclaimed underground natural gas pipeline ROWs on cropland. In this study, reclaimed underground natural gas pipeline ROWs of varying ages (TSR) required different fertilizer application rates to optimize crop yields. At the recommended fertilizer rate, crop DMY

in the 4-yr ROW soil was lower than that in the off-ROW soil, while DMY in the 10-yr ROW soil did not differ significantly from that in the off-ROW soil. The study also showed that crop yield in the 4-yr ROW soil became similar to the yield in the off-ROW soil when fertilizer nutrient application rate was increased to 150% of the recommended rates. The improvement in crop yield with higher fertilizer application rate could be due to an increase in plant nutrient availability in the ROW soil, as shown by nutrient uptake and N efficiency results. Given that a companion field study showed that crop yield losses persisted for 12 yr on the same ROWs from which our soils were sourced, findings from this growth room bioassay suggest that long-term yield losses on these reclaimed ROWs could be due to the impacts of active underground pipelines on the soil ecosystem. However, our findings showed the possibility of optimizing crop yields on reclaimed ROWs by increasing fertilizer application rates in the absence of pipeline operational effects on the soil ecosystem, such as on decommissioned pipelines. In addition, these results suggest the need to devise pipeline construction techniques which reduce heat transfer from the pipeline to the soil ecosystem. However, we recommend field-scale research to ascertain the effects of varying fertilizer rates on the productivity of reclaimed ROWs. Such studies should investigate more fertilizer application rates than those evaluated in this study and should be conducted on more sites that encompass a larger number of ROWs spanning a wider TSR range than tested in this study.

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6. OVERALL SYNTHESIS

6.1 Summary of findings and contributions to knowledge

The number and total length of underground oil and natural gas pipelines in Canada has been increasing in the last two decades (Canada's Oil & Natural Gas Producers, 2023). The pipeline inventories in other countries and regions show the same pattern as well (International Energy Agency, 2023). These trends are driven by the global increase in demand for oil and natural gas products (Bhaskaran et al., 2020; Gong, 2020). However, the continued construction of pipeline networks is increasingly threatening the size and quality of agricultural land. Therefore, there is a need to accurately quantify the impacts of underground pipeline construction on soil productivity and evaluate the recovery of productivity on reclaimed ROWs with increasing time. A review of current literature revealed several research gaps on the impacts of pipeline construction on soil and vegetation ecosystems and the influence of time on the recovery thereof. For example, many studies have been conducted on grasslands, wetlands, and forested land, whereas only a few studies have focused on agricultural lands, a handful of which have examined changes in soil health and soil microbial communities on reclaimed ROWs.

The field study presented in Chapter 2 is novel in several ways. The study evaluated the recovery of the soil ecosystem on reclaimed ROWs using soil health indicators. Soil health indicators have rarely been used to evaluate the reclamation of ROWs. The soil health indicators evaluated in this study (permanganate-oxidizable C (POXC), autoclaved citrate-extractable (ACE) protein, and soil respiration) are sensitive to changes in landuse (Culman et al., 2012; Schindelbeck et al., 2016; Wei et al., 2022), and therefore present the potential to evaluate the recovery of reclaimed ROWs with increasing time. In this study, soil respiration seemed to have recovered within 6 yr of ROW reclamation, whereas it took 12 yr for POXC and ACE protein to recover to

off-ROW levels. These results showed the potential of using soil health indicators to evaluate the recovery of reclaimed ROWs on cropland. This study is also among a few that have investigated the influence of TSR on the recovery of crop productivity on reclaimed ROWs on cropland in the Canadian prairies. Grain and biomass yields 12 yr post-reclamation were still 42% and 36%, respectively, lower on the ROWs than on adjacent off-ROWs, suggesting that the impacts of pipeline construction on crop productivity are long-term. We expected plant available nutrients to be lower on reclaimed ROWs than on off-ROWs and to increase with increasing TSR. However, plant-available nutrients did not show any consistent trends across the treatments. Changes in plant-available nutrient concentrations may have been confounded by the application of inorganic fertilizers on the ROWs. Thus, plant-available nutrients may not be a reliable barometer for the recovery of reclaimed ROWs on cropland.

Previous studies have shown that pipeline construction has long-term impacts on soil physical properties (e.g., Soon et al., 2000; Dessierud et al., 2010; Shi et al., 2015). These impacts have largely been attributed to the movement of construction vehicles (Tekeste et al., 2019). The study presented in Chapter 3 investigated post-reclamation changes in soil penetration resistance (PR), field saturated hydraulic conductivity (K_{fs}), near-saturated hydraulic conductivity (K_{Ψ}), soil macroporosity, and wet aggregate stability (WAS) on natural gas pipeline ROWs on cropland. The results showed that PR was greater on the 4.5 yr than on the off-ROW. However, PR did not differ significantly between the 6.5- and 12.5-yr ROWs and the adjacent off-ROWs. Penetration resistance reflects soil compaction. The size and number of macropores decrease with increasing compaction (Berli et al., 2004; Zhang et al., 2006). This may explain the low effective water conductive macroporosity on the 4.5-yr ROW relative to the off-ROW. Both K_{fs} and K_{Ψ} were lower on the ROWs than on the off-ROWs. However, K_{Ψ} seemed to have recovered earlier than

K_{fs} . Overall, these results indicate a significant but slow recovery in soil physical attributes with increasing TSR. These soil physical properties influence crop productivity. For instance, PR greater than 3 MPa restricts the growth and development of crop roots, which in turn reduce water and nutrient uptake by the roots, thereby impacting crop yield (Moraes et al., 2020; Vaz et al., 2011). However, just like soil health indicators and plant available nutrients, the changes in the soil physical properties presented in Chapter 3 could not fully explain the long-term crop yield loss observed on the reclaimed ROWs. Therefore, Chapter 4 focused on the evaluation of soil biological attributes to further elucidate the factors that might be contributing to yield loss on the reclaimed ROWs.

Soil microorganisms carry out and facilitate processes such as nutrient cycling, organic matter decomposition, and aggregate formation (Doran & Zeiss, 2000). Through these processes, the microorganisms influence soil productivity. Studies have shown that the construction of underground pipelines can negatively impact soil microbial communities (e.g., Soon et al., 2000; Schindelbeck and van Es, 2012; Gasch et al., 2016). However, these studies employed techniques with lower taxonomic resolution than the 16S rRNA and ITS techniques reported in Chapter 4. The results from the present study showed that pipeline construction has deleterious impacts on soil microbial communities. Bacterial and fungal community diversity indices on reclaimed ROWs improved with increasing TSR, closely matching those on the off-ROWs after 11-12 yr post-reclamation. The improvement in microbial diversity was positively correlated with soil attributes such as soil organic C, which are presented in Chapter 2. Time since reclamation also influenced bacterial and fungal community composition. However, the relative abundance of some bacterial and fungal phyla was greater on younger ROWs than on older ROWs and the off-ROWs, indicating

that different microorganisms play varying roles in the recovery of the soil ecosystem on reclaimed ROWs.

This research showed that crop yield losses persist for longer periods than the soil properties measured in this study. This suggests that there are other underlying factors limiting crop productivity on reclaimed ROWs beyond 12 yr, such as the influence of an operating pipeline on soil properties. We hypothesized that, in the absence of active pipeline effects, crop yields on reclaimed ROWs of different TSRs would be optimized by increasing the fertilizer application rate. This hypothesis was tested in a growth room bioassay using soils collected from reclaimed ROWs. The results showed that the fertilizer rate recommended for the off-ROW produced lower wheat and canola biomass yields on the 4- and 10-yr ROWs than on the off-ROW. Increasing the fertilizer application rate by 50% resulted in a significant increase in canola and wheat biomass yield on both the 4- and 10-yr ROWs such that the yield on the ROWs matched that on the off-ROW. These results suggest that, in the absence of the effects of an active pipeline, crop yields on reclaimed ROWs could be improved by increasing fertilizer application rate. This study lays out the groundwork for further research aimed at improving crop productivity on reclaimed ROWs.

Although the results presented in this thesis are not exhaustive, they show that crop yield losses on reclaimed ROWs (Chapter 2) could be partly due to increased PR and reduced hydraulic conductivity on the ROWs (Chapter 3), and the deleterious impacts of pipeline construction on components of the soil microbiome (Chapter 4). These results corroborate findings from previous studies (e.g., Culley & Dow, 1988; Soon et al., 2000; Shi et al., 2015). Pipeline construction has also been shown to negatively impact several other soil attributes, including soil temperature (Naeth et al., 1993; Halmová & Fehér, 2014) and soil moisture (Halmová et al., 2017), which were

not measured in this thesis research. It is possible that some of these attributes could be contributing to the observed crop yield depressions in ROWs of TSR of 12 yr or more.

The recovery of soil and crop variables with increasing TSR observed in this thesis could be attributed to the influence of agronomic practices and natural soil-building processes or their interaction. The addition of SOM from retained crop residues, the activity of crop roots of different architecture in crop rotations, and periodic tillage could all contribute to improvements in soil physical, chemical, and biological attributes, with eventual improvements in crop yield. For example, cropping systems that encourage the build-up of organic carbon, such as crop rotations, have been shown to promote soil microbial diversity and SOM accumulation on cropland (Esmailzadeh-Salestani et al., 2021). Similarly, the addition of organic matter during reclamation or as a land management strategy can accelerate the recovery of reclaimed land because organic matter improves aggregate formation and stability, soil porosity, and biological activity (Gasch et al., 2014; Larney and Angers, 2012). In addition, repeated crop rotations and periodic subsoiling have been shown to reduce soil compaction on cropland (Spoor, 2006). Farmers participating in this Ph.D. research employ these land management strategies, which have likely contributed to the observed improvements in soil properties and crop productivity with increasing TSR.

6.2 Implications of the research

Most of the previous studies that evaluated the productivity of reclaimed ROWs were conducted on non-agricultural ecosystems. This Ph.D. research is one of a few studies that have investigated the influence of TSR on the recovery of soil productivity on cropland. The research further investigated soil health and microbial communities on the reclaimed ROWs. These soil properties have not received much attention in previous studies. A key finding of this research was that the negative impacts of pipeline construction on soil productivity will gradually ameliorate

with increasing TSR. However, the impacts on crop yield seemed to persist longer than the soil properties measured in this research.

These findings are important to policymakers, reclamation specialists, pipeline construction companies, landowners, and researchers in formulating policies and guidelines on the construction and reclamation of pipeline ROWs and in developing land management strategies to restore productivity on reclaimed ROWs. In addition to the knowledge gaps identified in the literature review, this study was also necessitated by the growing concerns by landowners of persistent yield losses on reclaimed ROWs. This research showed that indeed yield losses on reclaimed ROWs are long-term (Chapter 2). This information will assist policymakers formulate policy frameworks on crop yield loss compensation. In addition, the findings will form a solid foundation on which compensation discussions and agreements between landowners and pipeline construction companies will be based, thus reducing possible conflicts and misunderstandings.

Pipeline construction seemed to have impacted soil health (Chapter 2), some soil physical properties (Chapter 3), and soil microbial communities (Chapter 4). Over the past three decades, pipeline construction and reclamation strategies that minimize ecological impacts, such as the “double-lifting” technique, have been developed. However, findings presented in this thesis suggest that more effective pipeline construction and reclamation techniques should be researched and developed. Information generated from this study is crucial to pipeline construction companies and reclamation specialists in evaluating their construction and reclamation techniques. In addition, this research identifies knowledge gaps for future studies on pipeline construction techniques that minimize impacts on the soil and vegetation ecosystems.

The soil health indicators evaluated in this study (POXC and ACE protein) could differentiate reclaimed ROWs of different TSR, showing their potential to be used in evaluating

reclamation success on ROWs on cropland. These findings are a significant addition to the “toolbox” of reclamation specialists and policymakers. The soil health indicators could be used to evaluate the recovery of reclaimed ROWs on cropland and possibly be scaled up to other ecosystems as well.

The growth room bioassay (Chapter 5) showed that crop yield on reclaimed ROWs could be improved to off-ROW levels by increasing fertilizer recommendation by 50% in the absence of operational pipeline effects. This information is crucial for farmers whose fields are traversed by natural gas pipelines as they seek strategies to optimize productivity on reclaimed ROWs. It is noteworthy that these findings are only applicable if crop productivity on reclaimed ROWs is limited by the presence of an operating pipeline. As such, this information could be useful for decommissioned pipelines on cropland.

6.3 Recommendations

Findings from this study showed that, with increasing time since reclamation, soil properties and crop productivity on reclaimed ROWs would converge towards values on adjacent undisturbed sites. However, this study was not exhaustive. Soil properties measured in the study seemed to have recovered within 12 yr of reclamation while crop yield losses persisted beyond 12 yr following ROW reclamation. This suggests that there are other factors still limiting crop productivity beyond 12 yr post-reclamation. Future studies should assess the critical effects of potentially elevated ROW temperatures on soil moisture dynamics and availability to plants. Elevated soil temperature on the ROWs could impact crop growth and productivity, particularly during drier years and in dry regions such as northeast British Columbia. In addition, I recommend further studies using model simulation to extend the estimation to incremental distances from the

pipeline centerline towards the off-ROW and exploring practical approaches to mitigate temperature elevation effects on the ROWs.

Although crop productivity on reclaimed ROWs improved with increasing TSR, there is a need to shorten the recovery period to minimize economic losses to landowners. Studies to determine best management practices that accelerate the recovery of ROWs on cropland are recommended. In this thesis, recovery of soil properties and crop productivity was attributed to agronomic practices such as crop rotations, retention of crop residues, and minimum soil disturbance, all of which enhance soil organic matter buildup. The influence of these agronomic practices on the recovery of reclaimed ROWs should be ascertained. Farmers in the study area do not grow cover crops; however, future studies could also evaluate the influence of cover crops on the recovery of reclaimed ROWs, since cover crops have been shown to be effective in enhancing soil health and building soil organic matter (e.g., Bolinder et al., 2020; Wulanningtyas et al., 2021).

Findings from the growth room bioassay (Chapter 5) showed that crop yields on reclaimed ROWs could be improved by increasing the fertilizer application rate by 50% in the absence of the influence of an operating pipeline. A complimentary field study is recommended to ascertain the effects of increasing fertilizer rate on crop yield on reclaimed ROWs. In addition, the field study should include several rates ranging between the recommended and 1.5 times the recommended rate so that the optimum rate could be determined. Such studies would immensely contribute to our understanding of the factors limiting crop yield on reclaimed ROWs. In addition, findings from such a study would contribute toward devising solutions to depressed crop yields on reclaimed ROWs.

Future studies should also scale-up this research project by increasing study sites (10-15 sites). These sites should include ALR lands with pipeline footprints varying widely in recovery periods

based on time of ‘Leave to Open’ filing. The sites would capture a cross section of ROWs constructed during frozen and unfrozen soil conditions (summer vs. winter). The selected pipelines would be sampled along a minimum of 800 m. We suggest three functional age categories: (1) pre-2013 (3 ages ranging from 11 to 25 years will be selected), (2) 2013-2017 (one age), (3) post 2017 (one age). Soil and crop samples would be taken from the ROW working side, trench line, and soil storage side, and from the off-ROW withing each field. In addition to measurements assessed in this PhD research project, these future studies could also incorporate other measurements such as soil temperature and moisture as suggested earlier.

6.4 References

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APPENDICES

I. Supplemental Information for Chapter 2

Table S2.1 Crops seeded at each of the three sites during the study period.

Sampling year	Seeded crop		
	Site 1	Site 2	Site 3
2020	Pea	Canola	Barley
2021	Barley/Oats	Wheat	Canola
2022	Canola	-†	-

†In 2022 Sites 2 and 3 were harvested before sampling could be done

Table S2.2 Nitrogen and phosphorus concentration in grain and straw as affected by pipeline ROW age.

Crop	Treatment	N concentration		P concentration	
	TSR †	Grain	Straw	Grain	Straw
		g kg ⁻¹ DMY			
Wheat	6 yr	21.3 a‡	9.7 a	3.72 a	0.65 a
	9 yr	-§	-	-	-
	12 yr	22.3 a*	.	2.37 b	-
	Off-ROW	23.3 a	10.4 a	3.61 a	0.63 a
	<i>P-value</i>	0.08	0.29	0.03	0.8
Oat	6 yr	25.6 a	14 a	3.23 a	1.2 a
	9 yr	26.1 a	12.7 a	3.25 a	1.03 a
	12 yr	-	-	-	-
	Off-ROW	26.1 a	13.1 a	3.03 a	0.83 a
	<i>P-value</i>	0.70	0.74	0.44	0.1
Barley	6 yr	24.0 a	12.3 a	4.04 a	1.21 a
	9 yr	20.8 ab	11.1 a	3.69 a	0.96 b
	12 yr	17.9 c	9.41 b	3.77 a	1.46 a
	Off-ROW	19.1 bc	9.81 b	3.6 a	0.99 b
	<i>P-value</i>	0.002	0.04	0.14	0.02
Field peas	6 yr	35.1 a	14.7 ab	2.8 a	0.73 b
	9 yr	33.6 a	15.1 a	3.1 a	0.93 a
	12 yr	34.5 a	13.3 b	3.2 a	0.9 a
	Off-ROW	34.7 a	13.4 ab	2.8 a	0.73 b
	<i>P-value</i>	0.56	0.04	0.24	0.02
Canola	6 yr	37.4 b	8.05 a	7.36 a	0.45 b
	9 yr	35.6 bc	7.69 bc	7.35 a	0.73 ab
	12 yr	42.2 a	13 a	7.88 a	1.70 a
	Off-ROW	34.6 a	6.39 c	7.28 a	0.48 b
	<i>P-value</i>	0.0001	0.0001	0.29	0.0362

†TSR, time since reclamation

‡Means in a column for each crop followed by the same letter are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)

§The 9 yr ROW was not seeded with wheat, while the 12 yr ROW was not seeded with oats during the sampling years

*N and P uptake for 12-yr was determined using grain concentration only

II. Supplemental Information for Chapter 4

Table S4.1 Effect of time since reclamation on the relative abundance of bacteria phyla with greater than 1% abundance in soil from reclaimed underground natural gas pipeline ROWs of varying ages.

	Site 1			Site 2			Site 3		<i>P-value</i>		
	6-yr	12-yr	Off-ROW	5-yr	11-yr	Off-ROW	4-yr	Off-ROW	Site 1	Site 2	Site 3
<i>Abundant phyla †</i>											
Acidobacteria	11.4 b‡	14.0 a	16.4 a	7.0 b	12.9 a	14.9 a	12.1 a	12.8 a	0.03	0.01	0.59
Actinobacteria	28.0 ab	23.8 b	30.5 a	31.6 a	30.0 a	25.2 b	28.6 a	29.9 a	0.01	0.01	0.58
Bacteroidetes	3.4 a	5.7 a	3.3 a	7.8 a	2.4 b	2.3 b	3.4 a	3.9 a	0.08	0.0003	0.61
Chloroflexi	3.2 b	4.6 a	4.6 a	3.7 a	4.4 a	3.5 a	2.6 b	5.2 a	0.02	0.10	0.03
Gemmatimonadetes	4.7 b	5.4 a	5.3 a	5.0 a	5.4 a	4.9 a	4.4 a	3.6 a	0.02	0.56	0.34
Planctomycetes	4.2 a	4.6 a	3.6 a	3.6 a	3.4 a	2.9 a	2.8 a	3.7 a	0.13	0.19	0.47
Proteobacteria	2.5 a	2.3 a	2.1 a	18.3 b	19.8 ab	21.5 a	2.8 a	2.5 a	0.24	0.03	0.22
Verrucomicrobia	7.5 a	7.1 a	6.4 a	3.4 c	7.8 b	12.9 a	6.7 a	3.1 a	0.54	0.001	0.22
Firmicutes	5.3 a	3.3 a	4.7 a	12.4 a	5.6 b	3.6 b	5.1 a	3.2 a	0.09	0.001	0.06
Nitrospirae	0.7 b	1.0 ab	1.3 a	0.5 b	1.4 a	1.4 a	0.1 a	0.3 a	0.01	0.03	0.47
WPS-1	1.3 a	1.2 a	1.2 a	1.3 a	0.6 a	1.2 a	1.8 a	1.7 a	0.94	0.28	0.83
Others	4.1 a	4.8 a	5.0 a	4.4 a	5.6 a	5.3 a	3.1 a	5.3 b	0.1	0.3	0.02
Unassigned	0.1 a	0.2 a	0.1 a	0.001	0.001	0.003	0.002	0.004	0.2	0.3	0.51

†Relative abundance of sequence means are greater than 1% in abundance

‡Means in a row within the same site followed by the same letter are not significantly different at $\alpha = 0.05$ according to Tukey's multiple comparison procedure

Table S4.2 Effect of time since reclamation on the abundance of fungal phyla with greater than 1% abundance on reclaimed underground natural gas ROW on cropland.

	Site 1			Site 2			Site 3		<i>P-value</i>		
	6-yr	12-yr	Off-ROW	5-yr	11-yr	Off-ROW	4-yr	Off-ROW	Site 1	Site 2	Site 3
<i>Abundant phyla</i> [†]											
Ascomycota	52.6 a	49.2 a	40.3 a	72.2 a‡	57.4 b	53.6 b	62.6 a	58.3 a	0.274	0.003	0.244
Basidiomycota	3.9 a	3.3 a	3.6 a	1.0 b	3.8 a	2.2 a	3.7 a	4.7 a	0.455	0.006	0.122
Monoblepharomycota	0.1 a	0.1 a	0.1 a	0.01 a	0.03 a	0.1 a	0.1 a	0.1 a	0.585	0.262	0.41
Mortierellomycota	1.2 a	3.3 a	1.8 a	3.1 a	5.0 a	1.6 a	0.9 a	1.1 a	0.074	0.078	0.685
Mucoromycota	1.9 a	5.9 a	1.3 a	1.5 a	0.8 ab	0.3 b	0.3 a	0.1 b	0.09	0.034	0.041
Other	9.1 a	7.2 a	14.1 a	3.7 a	3.8 a	4.4 a	7.2 a	10.3 a	0.409	0.741	0.116
Unassigned	31.1 a	31.0 a	38.8 a	18.3 b	29.0 a	37.7 a	25.2 a	25.4 a	0.478	0.011	0.944

[†]Relative abundance of sequence means are greater than 1% in abundance

[‡]Means in a row within the same site followed by the same letter are not significantly different at $\alpha = 0.05$ according to Tukey's multiple comparison procedure

Table S4.3 Correlation analysis of soil properties and microbial attributes.

Microbial attribute	Coefficient	SOC [†]	Nitrate-N	Olsen-P	AK	Ca	Mg	pH	EC
Chao1 Bacteria	R^2	0.042	-0.304	-0.702	-0.387	-0.143	0.006	-0.064	0.491
	P	0.847	0.149	0.000	0.062	0.505	0.979	0.767	0.015
Shannon Bacteria	R^2	0.051	-0.436	-0.658	-0.405	-0.112	-0.025	-0.017	0.227
	P	0.812	0.033	0.001	0.050	0.604	0.909	0.938	0.285
Chao1 Fungi	R^2	-0.205	-0.383	-0.520	-0.463	-0.271	-0.219	0.257	0.062
	P	0.337	0.065	0.009	0.023	0.201	0.303	0.225	0.773
Shannon Fungi	R^2	-0.499	-0.385	-0.524	-0.757	-0.663	-0.657	0.277	-0.248
	P	0.013	0.063	0.009	<.0001	0.000	0.001	0.189	0.243
Ascomycota	R^2	0.073	0.445	0.576	0.655	0.541	0.473	0.012	-0.039
	P	0.734	0.029	0.003	0.001	0.006	0.020	0.957	0.855
Basidiomycota	R^2	-0.109	-0.211	0.078	-0.428	-0.592	-0.607	0.245	-0.515
	P	0.612	0.322	0.718	0.037	0.002	0.002	0.250	0.010
Monoblepharomycota	R^2	-0.268	-0.156	-0.293	-0.433	-0.406	-0.405	-0.049	0.260
	P	0.206	0.467	0.164	0.035	0.049	0.050	0.822	0.220
Mortierellomycota	R^2	-0.036	-0.263	-0.001	0.200	0.284	0.282	0.142	-0.203
	P	0.866	0.215	0.996	0.349	0.179	0.182	0.507	0.342
Mucoromycota	R^2	-0.008	-0.180	-0.415	-0.111	0.248	0.240	-0.404	0.137
	P	0.970	0.401	0.044	0.607	0.242	0.258	0.050	0.524
Acidobacteria	R^2	-0.573	-0.205	-0.206	-0.647	-0.613	-0.726	0.124	-0.143
	P	0.003	0.338	0.335	0.001	0.001	<.0001	0.563	0.504
Actinobacteria	R^2	0.545	-0.009	0.449	0.528	0.240	0.320	0.231	-0.109
	P	0.006	0.966	0.028	0.008	0.259	0.127	0.277	0.613
Proteobacteria	R^2	-0.238	0.216	0.084	-0.405	-0.570	-0.593	0.150	-0.306
	P	0.263	0.312	0.698	0.050	0.004	0.002	0.484	0.145
Verrucomicrobia	R^2	-0.600	-0.394	-0.471	-0.515	-0.318	-0.310	-0.125	0.268
	P	0.002	0.057	0.020	0.010	0.130	0.140	0.560	0.205
Firmicutes	R^2	0.485	0.402	0.343	0.786	0.847	0.876	-0.209	0.200
	P	0.016	0.051	0.101	<.0001	<.0001	<.0001	0.327	0.349

[†]SOC, soil organic carbon; Olsen-P, available phosphorus; AK, available potassium; Ca, extractable calcium; Mg, extractable magnesium; EC, electrical conductivity

III. Supplemental information for Chapter 5

Table S5.1 Canola and wheat tissue N and P concentration as affected by crop phase, time since reclamation, and fertilizer application rate.

Effect	Concentration (g kg ⁻¹ DMY)			
	Canola		Wheat	
	N†	P	N	P
Phase				
1	27.4 a‡	3.4 a‡	29.7 a	2.8 a
2	15 b	2.8 b	15.7 b	2.7 a
3	15 b	3.5 a	13.3 b	2.3 b
TSR				
4	18.1 b	2.8 c	19.6 a	2.2 c
10	20.3 a	3.1 b	18.9 a	2.5 b
Off-ROW	18.9 b	3.9 a	20.3 a	3.1 a
Rate				
Control	17.4 b	3.3 a	16.5 c	2.7 a
100%	19.6 a	3.3 a	19.5 b	2.5 ab
150%	20.3 a	3.1 a	22.8 a	2.5 b
	<i>P-value</i>			
Phase (P)	<.0001	<.0001	<.0001	<.0001
TSR	0.02	<.0001	0.36	<.0001
Rate (R)	0.0001	0.13	<.0001	0.03
TSR × R	0.003	0.17	0.1083	0.001
TSR × P	0.95	<.0001	0.004	<.0001
Rate × P	0.001	0.02	0.23	0.05
TSR × R × P	0.4	0.01	0.38	0.05

†N, total nitrogen; P, total phosphorus; TSR, time since reclamation

‡Means followed by the same letter in a column under each effect are not significantly different according to Tukey's multiple comparison procedure ($\alpha = 0.05$)