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Adaptation strategies of forage soybeans cultivated on acidic soils under cool climate to produce high quality forage

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ABSTRACT

Boreal soils tend to be podzols characterized by acidic pH, which can further limit forage crop growth and production. It is unclear, how forage soybeans adopt to produce forage with high nutritional quality when cultivated on podzols in boreal climate. To answer this question, we cultivated forage soybeans on agricultural podzols at 3 farm sites with varied soil pH (6.8, 6.0 or 5.1), and assessed the root membrane lipidome remodeling response to such climatic conditions. Contrary to our expectations, significantly lower biomass was observed at pH 6.8 compared to 6.0 and 5.1. However, surprisingly the plants produced similar forage quality at 6.8 and 5.1 pH. Three major lipid classes including phospholipids, glycolipids and phytosterols were observed in roots irrespective of soil pH. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA), and acylated glucosyl betasitosterol ester (AGlcSiE) accounted for 95% of the root lipidome, and expressed significant changes in response to cultivation across the three soil pH levels. These lipids were also observed to have strong correlations with forage production, and forage quality. Therefore, soybean genotypes with higher abilities to remodel PC, PE, PA, and AGlcSiE could be better suited for producing higher quality forage in acid podzolic soils characteristics of boreal ecosystems.

1. Introduction

Farmers in boreal environments with cool climates have serious challenges to find agronomic crop varieties ideally suited to grow and produce a high quality crop to feed domestic livestock or human [1,2]. These regions experience late spring and early fall, whereas the short crop growth season usually has low temperatures during seedling establishment, and increase chances of frost damage near the crop maturity [3]. There is an urgent need to develop production systems that facilitate optimum crop productivity, as well as, permits the potential expansion of geographic distribution of more agronomic crop species for cultivation to enhance food security in boreal regions [4,5]. In addition to the challenges with low temperatures and short growing seasons; much of the soils in these regions tend to be shallow [2,6,7]. Boreal soils are predominately podzolic and are being converted for agricultural crop production; however, these soils are prone to high nutrient losses [6,8]. Podzolic soils are considered least favorable for agricultural crop production owing to lower fertility, potential toxicity from soluble forms of elements such as Al, Mn and Fe, unfavorable physical properties and acidic nature [2,9]. As such, crop adaptation strategies are critical to the success of crop production systems in the cool climatic conditions and acidic soils of boreal ecosystems [2].

Forage and grain crops have high capacity to acclimate and produce desirable harvest under the prevailing climatic and soil conditions atypical of boreal ecosystems. There is huge interest in the scientific community to develop crop production systems in which forage soybeans (*Glycine max* L.) are either intercropped or cultivated in rotation with silage corn as a source of high quality forage to meet the animal feed requirements [10,11] in northern climates. Soybeans are generally harvested at the R3 growth stage which is thought to be the best stage for preserving the forage's nutritional quality, while achieving optimal biomass with superior palatability [12,13]. Therefore, the addition of

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soybean into existing forage production systems would have several benefits such as; a source of protein rich forage, improves the soil nutrient composition by fixing nitrogen, and enhances the soil health status by improving the active microbial community structure and nutrient cycling [14–17]. However, there are challenges to finding suitable forage soybean genotypes with low crop heating unit (CHU) requirements ideally adapted or suitable for cultivations under boreal conditions. Geographic locations such as Newfoundland, Canada receive as low as 1800 CHU during the crop-growing season, whereas many soybean genotypes require at least 2250 CHU; particularly if the crop is desired to reach the full maturity [1]. Adaptation of the soybean cell membrane to low temperatures and acidic soils in these production systems are critical to the crop's successful response and survival.

The root plasma membranes play important roles in determining the growth and development of plants under a wide variety of environmental conditions [18,19]. For instance, the root plasma membrane is the site of complex sensors, that can detect variations in nutrient availability, soil pH, and water stress, as well as transduce signals inside the cells to distal portions of the plant to provide a rapid response to stressors [19–23]. Lipids are important components in this process, and play a critical role in determining the physicochemical properties of the membranes [24,25]. The root lipid composition is known to vary between plant species, plant organs or organelles, plant growth stages, and in response to the environmental growth conditions [18,26–28]. Soybean root membranes are composed of major lipids including; phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), as well as minor lipids such as phosphatidic acid (PA), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE) and lysophosphatidylglycerol (LPG) [23]. Additionally, root membrane lipids have been demonstrated to be remodeled in response to changes in the nutrient levels in the root zone [23]. For instance, a decrease in PE and a concurrent increase in PA were observed in response to nitrogen and phosphorus deficiencies in the growth media [23]. Although there is plenty of information available in the literature concerning changes in the soybean root lipidome under varying environmental conditions; there is a lack of information in the literature concerning how forage soybeans modulate root lipid composition to adapt, grow and produce a high nutritional quality forage when cultivated on agricultural podzols in boreal ecosystems. Understanding how the root plasma membrane lipidome is remodeled are paramount to our understanding of how plants perceive and respond to fluctuations to different soil pH under cool climatic growth conditions in boreal ecosystems. To address these issues and deficit in information, the current study was designed to investigate: i) how forage soybean root membrane lipidome is remodeled in response to cultivation on agricultural podzolic soils; ii) the effects of the remodeled root lipidome on forage production and nutritional quality; and iii) the possible relationships between the remodeled root membrane lipidome, forage production and nutritional quality when cultivated on agricultural podzolic soils in boreal ecosystems.

2. Materials and methods

2.1. Plant cultivation, soil properties and weather conditions

The research trial was conducted on three farms with different soil pH extended from east to west across the Province of Newfoundland, Canada (Fig. 1; Table 1). Soybean genotype (DEKALB RoundUp Ready 25-10RY) was obtained from Hillview Farms (New Brunswick, Canada), and was seeded @ 120 K seeds per acre with Western Great Plains no-till compact drill (606 N T Great Plains Manufacturing Inc. USA) on June 15, 2015. Crops were seeded at 19 cm row spacing on 1.5 m × 7.0 m plots in a completely randomize block design with three replicates on each site. Before seeding, pre-emergence herbicide (RoundUp WeatherMax™) followed by tillage and inorganic fertilizers were applied to fulfil the nutrient requirements. A mixed fertilizer (11-22-22 NPK, 5% sulfur, 0.3% boron) was added to the soil at the rate of 225 kg ha^{-1} keeping in view of the soil nutrient status, and regional soybean recommendation guidelines and the characteristics of study sites as reported in Table 1. Grass-legume mixture of silage or silage corn were grown in three farms during the previous three growing seasons. Of the three soil pH values, 6.8 was considered as the control since most of the minerals are available at pH 6.5–7.0 for plant uptake for optimum growth [29,30]. Weather data during the crop growing season was obtained from weather stations located on or adjacent to the farm sites and are reported fortnightly (Table 2).

2.2. Soybean forage quality analyses

A cohort of plants selected from 1 m^2 area was harvested by carefully uprooting at the R3 growth stage from each experimental plot. Rhizosphere soil was gently removed from the uprooted plant roots, and the plants were then separated into roots and shoots. The roots were then immersed immediately into liquid nitrogen and transferred in the laboratory for lipids analysis. Shoots from the same plants were hand cut into small pieces, pooled for each replication and dried at 60°C in a forced air oven (Shel Lab FX14-2, Sheldon Manufacturing Inc. USA) until a constant dry weight was achieved. The dried plant samples were then ground using a cryomill (Retsch GmbH Germany) and transferred to the Activation Laboratory (Ancaster ON, Canada), a member of the laboratory of Dairy One Feed and Forage Analyses Laboratories (Ithaca, New York USA) for forage quality analyses. Near Infrared Reflectance Spectroscopy technique (Foss NIRSystem Model 6500 Win ISI II v1.5) was used to measure dry matter (DM), forage proteins (crude protein: CP, available protein: AP, adjustable crude proteins: ACP, soluble proteins: SP, degradable proteins: DP, neutral detergent insoluble crude proteins: NDICP, acid detergent insoluble crude protein: ADICP), minerals (potassium: K, calcium: Ca, phosphorus: P, magnesium: Mg, sulfur: S), fiber (acid detergent fiber: ADF, neutral detergent fiber: NDF, in-vitro NDF digestibility at 30 h: NDFD30 h, lignin), starch, ash contents, simple sugars (SS), water soluble carbohydrates (WSC), crude fat and non-fibrous carbohydrates (NFC). Total digestible nutrients (TDN) were assessed using summative equation based on forage quality components at maintenance level 1x [31]. Forage energy (net energy for lactation: NEL, net energy for maintenance: NEM, net energy for gain: NEG), were calculated using National Research Council [32] standard equations and Van Soest variable discount approach [33]. Finally, the relative forage quality (RFQ) was assessed in forage based on different quality parameters.

2.3. Soybean forage production

One square meter area was harvested at the R3 stage from each experimental plot and the fresh weight was measured. The forage production per hectare was calculated on a DM basis for all three farms.

2.4. Soybean root lipid extraction

Root samples were incubated in boiling isopropanol and lipids analyzed according to our previously published methods [34–37].

2.5. Statistical analyses

Principal component analysis (PCA) and Heat map analyses were performed using XLSTAT (Premium 2017, Version 19.5; Addinsoft, Paris, France) to determine the relationships between soil pH, root lipids, forage production and/or forage nutritional quality. Analysis of variance (ANOVA) was used to determine the effects of soil pH on root lipid components, forage production and nutritional quality using Statistix-10 software package (Analytical Software, FL, USA). Where treatment effects were significant, the means were compared with Fisher's Least Significant Difference (LSD) at alpha = 0.05. Pearson's

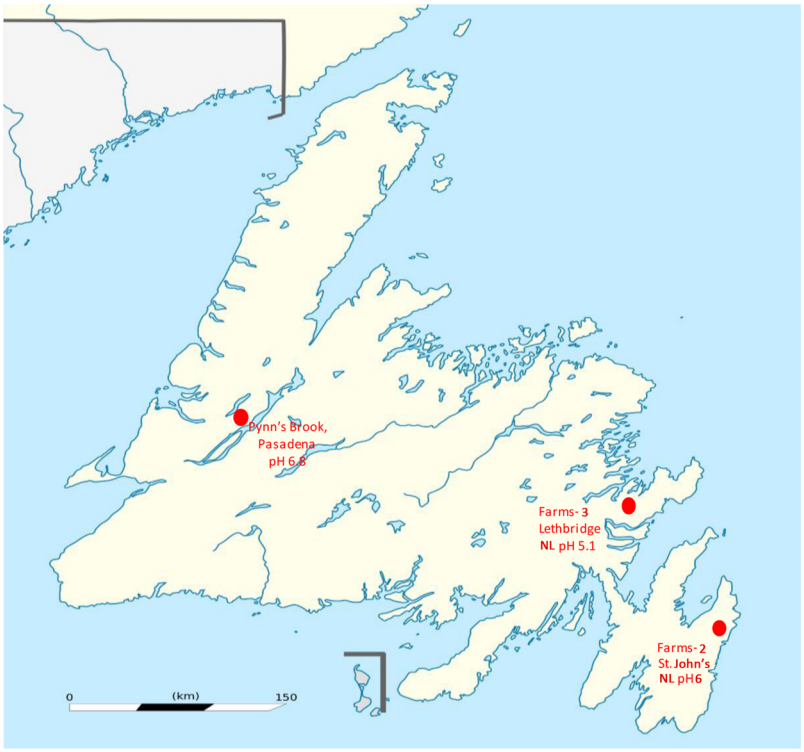

Fig. 1. Farm selection for current study at three geographical locations (Pynn's Brook Research Station, Lethbridge, and St. John's) across Newfoundland, Canada.

Table 1
Site locations and soil pH of the agricultural farms used for the soybean research study.

Farms	Field Locations	Soil pH	Coordinates
Farm-1	Pynn's Brook, NL	6.8	49° 4'21.93"N 57°33'36.51"W
Farm-2	St. John's, NL	6.0	47°30'4.99"N 52°46'12.34"W
Farm-3	Lethbridge, NL	5.1	48°20'21.72"N 53°49'24.80"W

correlation coefficient was used to test the relationship between root lipids, forage production and nutritional quality. Figures were prepared using SigmaPlot 13.0 software program (Systat Software Inc., San Jose, CA).

3. Results and discussion

3.1. Soybean forage production

The focus of this study was to determine how forage soybeans remodel root membrane lipids to adapt, grow and produce a forage crop with high nutritional quality when cultivated in acidic soils (agriculture podzol) under cool climatic conditions in boreal ecosystems. Contrary to our expectations, the study results revealed significantly lower forage biomass under field conditions at soil pH of 6.8 (2.18 ± 0.23 Mg ha^{-1}) compared to soil pH of 6.0 (3.55 ± 0.07 Mg ha^{-1}), and pH of 5.1 (3.59 ± 0.13 Mg ha^{-1}) [Fig. 3]. Results suggested that acidic soils (5.1 and 6.0) had enhanced forage production (39% and 38%, respectively) compared to the control (pH 6.8). The observed acid phosphatase activity was slightly lower at pH 6.8 compared to pH 6 and pH 5.1, though non-significant (Fig. 1, DIB [38]). The majority of crop plants prefer a soil pH range of 6.0–7.5, where most of the micro and macro nutrients become available to the plant roots in ample amounts [29,30]. However, podzolic soils common in boreal ecosystems with cool climates as observed at the study sites contain acidic soils, which can decrease availability of nitrogen (N), phosphorus (P), and other minerals [2,18]. Consequently, plants cultivated in pH 6.8 would be expected to produce significantly more biomass compared to those cultivated on 6.0 or 5.1 pH soils (Fig. 3a). However, considerations should also be given to other environmental factors such as the total precipitation (Table 2) in explaining the lower forage production. The

Table 2
Fortnightly weather growth conditions during the soybean growth season across the three study sites. Maximum temperature (T_{max} °C), minimum temperature (T_{min} °C), mean temperature (T_{mean} °C), and rainfall (RF mm) at farm-1 (soil pH 6.8), farm-2 (soil pH 6.0), and farm-3 (soil pH 5.1) during the 2015 crop growing season.

Growth period	pH 6.8				pH 6.0				pH 5.1			
	T_{max}	T_{min}	T_{mean}	RF	T_{max}	T_{min}	T_{mean}	RF	T_{max}	T_{min}	T_{mean}	RF
June 1-15	22.3	0.3	11.3	53	13.3	3.3	8.5	55	15.6	4.7	10.2	49
June 16-30	21.3	5.1	13.2	26	15.4	5.9	10.7	83	18.0	6.5	12.2	30
July 1-15	23.6	9.8	16.7	21	17.4	5.6	12.8	96	19.9	9.5	14.7	33
July 16-31	21.2	9.4	15.3	79	14.2	8.7	11.2	86	16.2	9.3	12.7	61
August 1-15	25.3	8.6	16.9	17	21.7	12.7	17.4	32	24.1	12.3	18.2	47
August 16-31	26.4	14.1	20.2	9	21.2	13.6	17.3	19	24.2	14.3	19.3	25
September 1-15	22.5	8.9	15.7	52	18.0	8.7	13.5	99	18.5	7.9	13.3	54
September 16-30	25.4	0.5	12.9	2	17.3	7.4	11.0	65	18.2	7.4	12.8	9
				259				**534**				**307**

plants cultivated on the 6.8 pH received 52% and 16% less rainfall compared to those cultivated on 6.0 and 5.1 pH, respectively (Table 2). Under normal growth conditions, soybean crop uses less water during the early growth stages, whereas the crop water requirements increases significantly during rapid vegetative growth [39–41]. As such, soybean crops require 450–700 mm water during growth season for optimum growth [42]. The soybean plants at the 5.1 pH received a maximum precipitation of 534 mm compared to 259 mm at the 6.8 pH, demonstrating plants at the 6.8 pH site received less than the recommended precipitation during the growing season [42]. Additionally, the precipitation patterns were quite different at the 6.8 pH, when the crop was approaching the pod formation growth stage (Table 2). The rainfall was well distributed throughout the growth season at 6.0 and 5.1 pH; whereas in addition to low precipitation, there were only three major rainfall events (> 50 mm) plus two events where the rainfall was less than 10 mm at the 6.8 pH site (Table 2). Soybean crop is very sensitive to water stress during mid and late reproductive growth, and any water stress could result in significant yield losses [41,42]. Therefore, significantly lower rainfall during the late growing period might have accounted for the 38–39% reduction in soybean forage production observed at the 6.8 pH compared to 6.0 and 5.1 pH sites (Fig. 3a). Consistent with the objective of this study, it will be interesting to see how the nutritional quality of the forage compared across these sites with varying pH, considering there are obvious differences in rainfall patterns.

3.2. Forage nutritional quality following cultivation on acidic soils (podzols) in boreal environment

Alfalfa is considered as the most nutritious forage with the highest quality among forage species [43–45]. It is also used as a reference crop to evaluate the energy values in other forage species [46]. Interestingly, we observed that plants produced significantly higher or similar nutrients content (forage quality) when cultivated in acidic soil (5.1) compared to the control soil pH (6.8) (Table 3). A total of 27 forage nutritional quality parameters were tested to determine the soybean forage quality and include: proteins, minerals, fibers, carbohydrates, crude fat, forage energy and relative feed values. The following are considered the most important indicators of forage nutritional quality [45,47–50]: crude proteins (CP), available proteins (AP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrient (TDN), net energy for maintenance (NEM), net energy for gain (NEG), net energy for lactation (NEL), and relative forage quality (RFQ). We observed 74% of the evaluated nutritional quality parameters were significantly higher or quantitatively similar to that of the soybean grown at 6.8 and 5.1 soil pH (Table 3). High forage protein content is one of the most desirable components in forage quality. In order to qualify as a good quality forage, the amounts of CP must be higher than 7% to guarantee adequate N supply for effective rumen microbial fermentation [51–53]. We observed that the CP values ranged between 19.86 — 27.7%, and the quantities were similar in soybeans cultivated at pH of 6.8 and pH of 5.1 (Table 3). These CP values are consistent with those reported in the literature for high quality alfalfa forage [43,44,54]. Similarly, the ACP values were not significantly different between the soybean plants cultivated in the acidic soil (5.1) compared to those cultivated at the control site. The ACP refers to portions of the forage N that is chemically linked to the forage fiber and is nutritionally unavailable for digestion [44,54]. The ACP rather than the CP is recommended to be used by animal nutritionists when formulating a balanced ration. Our results demonstrate that forage cultivated on the control and acidic soil produced similar levels of nutritionally unavailable proteins, which is further confirmed by the NDICP content (Table 3). Like the ACP, the NDICP gives an estimate of the unavailable fiber bound N [54]. All other measures of protein content in the forage were non-significant across the varying soil pH used for crop cultivation in this study (Table 3).

Like the CP, the fiber content is also of prime importance in forage quality and can be divided into ADF and NDF. The ADF refers to the least digestible portion of the roughage and consisted of cellulose, lignin, insoluble ash, cutin and pectin [54]. As a good forage quality indicator, ADF is inversely correlated with digestibility, and is used to estimate the energy values or digestibility of forage in animal feed formulation [55,56]. Forage containing 26—34% ADF is considered to be of good quality with high digestibility [54,57]. In the current study, we observed that ADF values ranged between 25.5 — 28.1%, indicating regardless of the soil pH, forage soybeans acclimate and produced similar high-quality forage as plants cultivated at pH 6.8 under cool climatic conditions (Table 3). These results suggest that soybean grown in boreal ecosystems on podzolic soils across a range of acidic soil (5.1 — 6.0) without liming can produce forage with similar nutritional quality and digestibility as those produced at neutral pH. It is accepted in the scientific community that good quality forage with high digestibility must contain less than 50% NDF [54,57]. We observed that the NDF values fluctuated between 32.0 — 33.9%, and that there is no significant difference in the NDF content of the crop cultivated in the soils with different pH (Table 3). The NDF is used to predict the intake potential of the forage or animal feed, as well as, for forage energies [47,56,58]. Collectively, these findings demonstrated that soybeans harvested at the R3 stage consistently produced forage with similar nutritional quality and feed intake potential, when cultivated on soils with different pH in boreal ecosystems. TDN represent the sum of CP, digestible fat, digestible NFC, and NDF typically used as a measure of forage energy and digestibility [47,50,59]. TDN value equal or greater than 65% is indicative of forage with superior quality and nutritive value [60]. TDN values obtained in the current study ranged between 65.66 — 66.67% across all the soil pH tested, suggesting the forage produced even on the acidic soil had high nutritive values (Table 3). In all cases, the observed TDN values were above the threshold value for low quality forages [60,61]. Several measures are used to calculate the digestible energy estimates or energy value of a feed material used in animal nutrition [50,62], and include the NEM, NEG or NEL. NEM is an estimate of the energy value of forage or feed used to keep an animal in energy equilibrium [56]. NEG, on the other hand, is an estimate of the energy required for body weight gain above or beyond what is required for body weight maintenance. Whereas NEL is an estimate of forage energy used for maintenance plus milk production during lactation, inclusive of the last two months of gestation [47,56]. Consequently, all three energy parameters are important indices used to estimate the forage quality in the context of total energy of the forage to enhance animal performance [47,56]. Consistent with the results of the other feed quality parameters evaluated, the net energy values of the soybean forage (NEM, NEG, NEL) produced in the acidic soil were similar (not significant) to that observed in the forage produced at control soil pH (Table 3). Furthermore, the NEM, NEG and NEL values are well above the optimum limits observed in high quality alfalfa forage [56,63], suggesting the soybean forage grown under cool climatic conditions at acidic soil is of superior quality. This superiority in forage nutritional quality can be further ascertained by the RFQ values observed in the current study (Table 3). Alfalfa is considered the gold standard for high quality forage, and RFQ values ranging between 100 — 200 is indicative of a high quality alfalfa forage [54]. RFQ values observed in this study were between 213.33 — 223.33, and above the range indicative of superior forage quality in Alfalfa [54]. Taken all together, the forage quality parameters measured indicated that forage cultivated at acidic soil (6.0 and 5.1) under cool climatic conditions were able to adapt to the variations in the root growth environments and produced a forage crop of similar nutrient quality compared to the crop cultivated at control soil pH (Table 3). These findings suggest that the soybean root membrane was able to perceived the variation in acidity in the root zone, and mount an appropriate response strategy permitting survival and production of a superior nutritional quality forage [54].

Table 3
Nutritional quality indicators of soybean forage grown on three agricultural farms with different soil pH under cool climate production systems.

Soybean forage quality indicators		Soil pH 6.8 (control)	Soil pH 6	Soil pH 5.1
Proteins	Crude protein (CP, % DM)*	22.0 ± 1.12[b]	27.7 ± 0.92[a]	19.86 ± 1.31[b]
	Available proteins (% DM)[ns]	20.76 ± 1.01	21.85 ± 1.58	19.95 ± 0.14
	Adjustable crude proteins (% DM)*	21.80 ± 0.93[b]	27.05 ± 0.84[a]	19.86 ± 1.31[b]
	Soluble proteins (% CP) [ns]	46.33 ± 1.20	42.66 ± 1.76	40.0 ± 1.73
	Degradable proteins (% CP)[ns]	69.00 ± 1.20	69.00 ± 3.21	64.00 ± 1.53
	Neutral detergent insoluble crude protein (% DM)*	2.43 ± 0.38[b]	5.60 ± 0.64[a]	4.83 ± 0.23[a]
	Acid detergent insoluble crude protein (% DM)[ns]	0.97 ± 0.48	1.56 ± 0.07	1.33 ± 0.17
Minerals	Potassium (% DM)*	2.46 ± 0.03[a]	2.20 ± 0.07[b]	2.64 ± 0.07[a]
	Calcium (% DM)*	1.58 ± 0.03[a]	1.37 ± 0.01[b]	1.26 ± 0.02[c]
	Phosphorus (% DM)[ns]	0.36 ± 0.01	0.37 ± 0.01	0.41 ± 0.01
	Magnesium (% DM)*	0.48 ± 0.01[a]	0.33 ± 0.01[b]	0.35 ± 0.00[b]
	Sulfur (% DM)*	0.25 ± 0.01[b]	0.34 ± 0.01[a]	0.34 ± 0.00[a]
Fiber	Acid detergent fiber (% DM)[ns]	28.1 ± 0.98	25.93 ± 0.82	25.53 ± 1.10
	Neutral detergent fiber (% DM)[ns]	33.86 ± 1.64	32.03 ± 1.08	33.76 ± 2.45
	Lignin (% DM)[ns]	4.80 ± 1.17	6.26 ± 0.49	6.33 ± 0.44
	In-vitro NDF digestibility 30 h (% DM)*	57.33 ± 1.20[a]	43.67 ± 2.33[b]	42.33 ± 1.45[b]
WS carbs, ash, starch, crude fat	Total digestible nutrient (% DM)[ns]	65.66 ± 0.33	66.33 ± 0.33	66.67 ± 1.20
	Starch (% DM)*	9.7 ± 0.77[a]	3.4 ± 0.46[c]	6.26 ± 0.67[b]
	Ash (% DM)[ns]	8.85 ± 0.31	8.28 ± 0.31	7.88 ± 0.28
	Simple sugar (% DM)*	7.47 ± 0.43[b]	9.20 ± 1.05 [b]	12.43 ± 0.62[a]
	Water soluble carbohydrates (% DM)[ns]	9.13 ± 0.35	7.73 ± 0.98	10.53 ± 0.57
	Crude fat (% DM)[ns]	3.4 ± 0.36	2.93 ± 0.33	3.46 ± 0.32
	Non-fibrous carbohydrates (% DM)[ns]	33.57 ± 0.83	37.07 ± 1.18	39.67 ± 2.11
Energy and forage quality	Net energy for maintenance (Mcal kg^{-1} DM)[ns]	1.50 ± 0.03	1.51 ± 0.01	1.53 ± 0.05
	Net energy for gain (Mcal kg^{-1} DM)[ns]	0.91 ± 0.03	0.92 ± 0.00	0.93 ± 0.05
	Net energy for lactation (Mcal kg^{-1} DM)[ns]	1.55 ± 0.02	1.57 ± 0.01	1.58 ± 0.04
	Relative forage quality[ns]	214.67 ± 20.79	223.33 ± 4.97	213.33 ± 20.36

3.3. Forage soybean root membrane lipidome following field cultivation in acidic soil (podzol)

Analysis of the forage soybean root lipids following cultivation in acidic soil under cool climatic conditions in boreal ecosystem revealed fifteen lipid classes, irrespective of soil pH (Fig. 4). The heat map output demonstrated two distinct clusters of the soybean root membrane lipids according to the soil pH used for cultivation (Fig. 4). The root membrane lipids in group 1 is composed of beta sitosterol (SiE), monogalactosyldiacylglycerol (MGDG), acylated glucosyl beta-sitosterol ester (AGlcSiE), phosphatidic acid (PA) and phosphatidylethanolamine (PE), while the lipids clustered in the second group consisted of cardiolipin (CL), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), digalactosyldiacylglycerol [DGDG] (Fig. 4). Previous results obtained from seven day old grain soybean roots indicated the root membrane lipids were composed mainly of phospholipids and galactolipids; and that higher amounts of PC, PE and PI predominate with DGDG, MGDG, PG, Lyso-PG (lysophosphatidylglycerol), LPC, PS and PA occurring as minor components [23]. However, following cultivation under cool climatic conditions and on acidic soil (podzols), we observed the forage soybean root membrane lipidome was composed of ten phospholipids [PE, PA, PC, PG, PS, LPC, PI, CL, LPE (lysophosphatidylethanolamine), and LPG]; two phytosterols [SiE, and AGlcSiE]; and three glycolipids [DGDG, monogalactosyldiacylglycerol (MGDG), and sulfoquinovosyl diacylglycerols (SQDG)]. The root membrane lipids varied in order of PE > PA > PC > AGlcSiE > PG > PS > LPC > SiE > PI > DGDG > CL > MGDG > LPE > SQDG > LPG, irrespective of soil pH (Table 1, DIB [38]). However, this composition is in contrast to that observed in seven day old soybean root membrane [23]. Additionally, four major lipid classes (PE, PA, PC and AGlcSiE) accounted for 95% of the total root lipid profile, regardless of soil pH. PE was the major lipid class observed in the root membranes and increased 20% and 13% in plant roots when grown at soil pH 6 and 5.1, respectively (Fig. 5). Further detailed analysis revealed that PE was composed of eight molecular species including PE32:1, PE32:2, PE34:2, PE34:3, PE34:4, PE36:2, PE36:4, and PE37:4; and that there was significant quantitative differences in PE molecular species in response to cultivation in the acidic soil (Table 4). In plants cultivated on acidic soils (5.1 and 6.0), we observed a significant increase in monounsaturated PE molecular species including PE16:1/16:1 and PE18:1/18:1 (Fig. 6). Conversely, linolenic acid enriched PE molecular species (e.g. PE16:0/18:3 and PE16:1/18:3) were significantly reduced in the roots cultivated at pH 5:1 (Fig. 6). Consistent with reports in the literature, plants cultivated in low temperature have increase polyunsaturated fatty acids in the membrane as a strategy to mitigate cold temperature stress [64,65]. We observed that 83.99–86.29 nmol% of the forage soybean root PE molecular species were polyunsaturated, and the remaining 13.71–16.01 nmol% were present as monounsaturated fatty acids across all three soil pH (Table 4). PA was the second major lipid observed in root membranes irrespective of the soil pH, and increased 57% and 17% at soil pH 6 and 5.1 compared to control (Fig. 4). PA is known to be increased in membranes in response to a wide variety of environmental stresses including mineral deficiencies, temperature changes and pathogens infections [21,23,66,67]. Root PA content was observed to decrease in response to low soil acidity, but was only significantly different at pH 6.0. Further analysis of PA to determine the molecular species composition showed six individual molecular species including PA34:2, PA34:3, PA36:2, PA36:4, PA36:5 and PA36:6 (Table 5). PA34:2 (16:0/18:2) was the predominant molecular species ranging from 29.31 to 42.92 nmol%. Approximately, 50% of the PA molecular species were quantitatively the same in the roots at soil pH 6.8 and pH 5.1 (Table 5). Furthermore, all the observed PA molecular species were polyunsaturated possibly due to the low growth temperatures as suggested by other researchers [64,65].

PC was the third major phospholipid observed in the forage soybean root membranes, irrespective of soil pH, and interestingly decreased in root membranes up to 79% and 36% at soil pH 6 and 5.1 compared to pH 6.8 (Fig. 4). Eight (8) PC molecular species were observed with PC36:2 being the most abundant [43.44 — 63.47 nmol%] in forage soybean roots (Table 6). Like PC, the majority of the PE molecular species were polyunsaturated ranging from 77.13 — 83.32 nmol%, while the monounsaturated (19.39 — 21.66%) and saturated (0.29 — 4.48%) molecular species were present as minor components (Table 6). Increased lipid unsaturation is known to favor lower growth temperatures in the growth medium [68–71].

Acylated glucosyl beta-sitosterol ester (AGlcSiE) was the fourth major lipid class recorded in forage soybean root membranes when grown under cool climate, irrespective of soil pH. Interestingly, greater

Table 4
The effects of soil pH on forage soybean root membrane phosphatidylethanolamine (PE) molecular specie's composition following field cultivation under cool climatic conditions.

m/z $[M+H]^+$ ion	m/z $[M-H]^-$ ion	Molecular species	Diacyl species	pH 6.8	pH 6	pH 5.1
690.507	688.492	PE(32:1)^{ns}	16:0/16:1	16.01 ± 0.93	13.71 ± 0.08	13.79 ± 1.08
688.491	686.477	PE(32:2)*	16:1/16:1	13.05 ± 0.37^b	26.75 ± 0.33^a	11.33 ± 0.71^b
716.522	714.507	PE(34:2)^{ns}	16:0/18:2	15.18 ± 0.29	17.61 ± 1.88	19.66 ± 1.67
714.507	712.492	PE(34:3)*	16:0/18:3	22.96 ± 1.19^a	21.54 ± 0.62^a	13.71 ± 0.54^b
712.491	710.476	PE(34:4)*	16:1/18:3	13.73 ± 1.62^a	13.12 ± 0.61^a	6.34 ± 0.33^b
744.554	742.539	PE(36:2)*	18:1/18:1	16.94 ± 1.67^b	6.57 ± 0.74^c	34.12 ± 3.61^a
740.522	738.508	PE(36:4)^{ns}	18:2/18:2	0.39 ± 0.02	0.38 ± 0.04	0.53 ± 0.09
758.569	756.555	PE(37:2)^{ns}	19:1/18:1	1.74 ± 0.59	0.29 ± 0.03	0.50 ± 0.048
Total%				**100**	**100**	**100**
ΣSat				**–**	**–**	**–**
ΣMonounsat ^{ns}				**16.01 ± 0.93**	**13.71 ± 0.08**	**13.79 ± 1.08**
ΣPolyunsat ^{ns}				**83.99 ± 4.92**	**86.29 ± 0.08**	**86.21 ± 1.08**
Sat/Unsat				**0.00**	**0.00**	**0.00**

Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the same row accompanied by different superscripts are significantly different between three different soil pH at alpha 0.05. PE represents phosphatidylethanolamine, Monounsat = monounsaturated lipids, Polyunsat = polyunsaturated lipids. Precursor ions $[M+H]^+$ and $[M-H]^-$ were detected in positive and negative ion mode following C30-RPLC chromatography, respectively. Their fatty acid composition (diacyl species) were identified based on the fragmentation. The lipid components in the table are arranged based on the molecular species composition with the large number before the colon representing total number of carbons, while the numbers after the colon representing the total number of double bonds (eg 32:1 = 32 carbons with 1 double bonds).

changes were observed in AGlcSiE compared to PE, PA and PC (Fig. 5). AGlcSiE increased 184% and 100% in root membranes when grown at soil pH 6 and 5.1, respectively compared to control (Fig. 5). Significantly, lower AGlcSiE was observed in crops cultivated in soil pH 6.8, whereas higher values were recorded in soybean roots when grown at soil pH 6. Detailed analysis of AGlcSiE revealed seven molecular species were present in roots with AGlcSiE 16:0 (48.83–57.54%), AGlcSiE 18:1 (13.25–21%) and AGlcSiE 18:3 (7.53–13%) representing the major molecular species (Table 7). The majority of the AGlcSiE species (62.29–76.09%) were observed to be saturated, whereas the remaining molecular species were either monounsaturated or polyunsaturated (Table 7). The sat/unsat ratios increased significantly as the soil pH decreased (Table 7). In plants cultivated on acidic soil (5.1 and 6), we observed a significant decrease in monounsaturated and polyunsaturated species (AGlcSiE18:1, AGlcSiE18:2; AGlcSiE18:3) occurred concomitant with a corresponding increase in saturated (AGlcSiE16:0; AGlcSiE18:0; AGlcSiE24:0) molecular species compared to the control soil pH (6.8) [Fig. 6]. Only saturated AGlcSiE molecular species (AGlcSiE24:0) was observed to increase in plants cultivated on acidic soil. The remaining eleven root lipid classes accounted for approximately 5% of the total root lipidome, irrespective of soil pH (Fig. 4). In all cases, each of the minor lipid class accounted for less than 2% of the total root lipid composition at each soil pH (Fig. 4). To the best of our knowledge, this is the first report in the literature of forage soybean root lipidome following cultivation in field conditions in northern climates or boreal environments.

3.4. How soybean plant produced high quality forage in acidic soil under cool climate

The focus of the current study was to determine how forage soybeans modulate their root membrane lipid composition to adapt, grow and produce a high nutritional quality forage crop when cultivated in acidic soil (podzols) in boreal ecosystems. The lipids in plant cell membranes have been recognized as having significant mechanical defense mechanisms, as well as signal transduction roles in plasma membranes acclimation to environmental constraints or stressors [24,25,67,72–74]. The four major lipid classes (PC, PE, PA and AGlcSiE) observed in our study accounted for approximately 95% of the total root lipidome; where PC, PE and AGlcSiE are structural

Table 5
The effects of soil pH on forage soybean root membrane phosphatidic acid (PA) molecular specie's composition following field cultivation under cool climatic conditions.

m/z $[M+NH_4]^+$ ion	m/z $[M-H]^-$ ion	Molecular species	Diacyl species	pH 6.8	pH 6	pH 5.1
690.507	671.466	PA(34:2)^{ns}	16:0/18:2	29.31 ± 1.41	38.97 ± 1.24	40.92 ± 4.66
688.491	669.450	PA(34:3)*	16:0/18:3	15.88 ± 0.94^b	23.29 ± 0.19^a	13.55 ± 0.58^b
718.538	699.497	PA(36:2)*	18:0/18:2	8.90 ± 0.66^b	4.44 ± 0.94^c	18.88 ± 1.49^a
714.507	695.466	PA(36:4)*	18:2/18:2	30.7 ± 1.53^a	18.42 ± 0.15^b	16.53 ± 1.51^b
712.491	693.450	PA(36:5)^{ns}	18:3/18:2	12.92 ± 2.69	12.42 ± 0.18	8.55 ± 0.96
710.476	691.434	PA(36:6)*	18:3/18:3	2.92 ± 0.03^a	2.46 ± 0.35^a	1.60 ± 0.25^b
Total%				**100**	**100**	**100**
ΣSat				**–**	**–**	**–**
ΣMonounsat				**–**	**–**	**–**
ΣPolyunsat				**100**	**100**	**100**
Sat/Unsat				**–**	**–**	**–**

Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the same row accompanied by different superscripts are significantly different between three agricultural farmlands with different soil pH at alpha 0.05. PA represents phosphatidic acid, Sat = saturated lipids, Monounsat = monounsaturated lipids, Polyunsat = polyunsaturated lipids, Sat/Unsat: ratio between saturated and unsaturated lipids. Precursor ions $[M+NH_4]^+$ and $[M-H]^-$ were detected in positive and negative ion mode following C30-RPLC chromatography, respectively. Their fatty acid composition (diacyl species) were identified based on the fragmentation. The lipid components in the table are arranged based on the molecular species composition with the large number before the colon representing total number of carbons, while the numbers after the colon representing the total number of double bonds (eg., 34:3 = 34 carbons with 3 double bonds).

Table 6
The effects of soil pH on forage soybean root membrane phosphatidylcholine (PC) molecular specie's composition following field cultivation under cool climatic conditions.

m/z $[M+H]^+$ ion	m/z $[M+HCOO]^-$ ion	Molecular species	Diacyl species	pH 6.8	pH 6	pH 5.1
732.554	766.544	PC(32:1)^{ns}	18:1/14:0	1.77 ± 0.36	3.02 ± 1.08	3.88 ± 0.79
762.601	806.591	PC(34:0)*	18:0/16:0	0.29 ± 0.02^b	4.48 ± 1.37^a	0.49 ± 0.07^b
760.585	804.576	PC(34:1)^{ns}	16:0/18:1	12.26 ± 1.38	9.36 ± 1.47	15.56 ± 3.00
758.569	802.560	PC(34:2)^{ns}	16:1/18:1	8.59 ± 2.65	26.21 ± 7.09	10.93 ± 2.13
774.601	818.591	PC(35:1)^{ns}	19:1/16:0	2.00 ± 0.40	3.83 ± 1.67	1.48 ± 0.12
788.616	832.607	PC(36:1)*	18:0/18:1	0.35 ± 0.04^b	2.17 ± 0.58^a	0.73 ± 0.03^b
786.601	830.591	PC(36:2)^{ns}	18:1/18:1	62.33 ± 5.25	43.44 ± 8.86	63.47 ± 5.43
800.616	844.607	PC(37:2)^{ns}	19:1/18:1	12.40 ± 2.31	7.48 ± 1.89	3.45 ± 0.65
Total%				**100**	**100**	**100**
ΣSat*				**0.29 ± 0.02^b**	**4.48 ± 1.37^a**	**0.49 ± 0.07^b**
ΣMonounsat ^{ns}				**16.39 ± 1.08**	**18.38 ± 2.43**	**21.66 ± 3.02**
ΣPolyunsat ^{ns}				**83.32 ± 1.09**	**77.13 ± 3.36**	**77.85 ± 2.96**
Sat/Unsat				**0.00**	**0.04 ± 0.01**	**0.00**

Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the same row accompanied by different superscripts are significantly different among three different soil pH at alpha 0.05. PC represents phosphatidylcholine. Precursor ions $[M+H]^+$ and $[M+HCOO]^-$ were detected in positive and negative ion mode following C30-RPLC chromatography, respectively. Their fatty acid composition (diacyl species) were identified based on the fragmentation. The lipid components in the table are arranged based on the molecular species composition with the large number before the colon representing total number of carbons, while the numbers after the colon representing the total number of double bonds (eg., 34:2 = 34 carbons with 2 double bonds).

Table 7
The effects of soil pH on forage soybean root membrane acylated glucosyl betasitosterol ester (AGlcSiE) molecular specie's composition following field cultivation under cool climatic conditions.

m/z $[M+NH_4]^+$ ion	Molecular species	pH 6.8	pH 6	pH 5.1
832.70	AGlcSiE(16:0)*	48.83 ± 0.72^b	50.02 ± 1.79^b	57.54 ± 1.45^a
860.73	AGlcSiE(18:0)*	9.61 ± 0.30^b	16.94 ± 1.68^a	12.79 ± 0.24^b
858.72	AGlcSiE(18:1)*	3.71 ± 0.25^a	1.45 ± 0.26^b	3.12 ± 0.09^a
856.70	AGlcSiE(18:2)*	21.00 ± 0.35^a	13.80 ± 0.79^b	13.25 ± 1.35^b
854.68	AGlcSiE(18:3)*	13.00 ± 0.35^a	10.95 ± 0.57^b	7.53 ± 0.29^c
916.79	AGlcSiE(22:0)*	4.16 ± 0.11^a	2.06 ± 0.09^b	2.09 ± 0.21^b
944.83	AGlcSiE(24:0)*	2.40 ± 0.27^c	4.78 ± 0.27^a	3.67 ± 0.02^b
Total%		**100**	**100**	**100**
ΣSat*		**62.29 ± 0.65^b**	**73.80 ± 0.90^a**	**76.09 ± 1.47^a**
ΣMonounsat*		**3.71 ± 0.25^a**	**1.45 ± 0.26^b**	**3.12 ± 0.09^a**
ΣPolyunsat*		**33.99 ± 0.58^a**	**24.75 ± 1.10^b**	**20.78 ± 1.48^b**
Sat/Unsat*		**1.65 ± 0.79^c**	**2.82 ± 0.66^b**	**3.18 ± 0.93^a**

Values (nanomole percent by weight composition) represent means ± standard errors for four replicates. Means in the same row accompanied by different superscripts are significantly different among three different soil pH at alpha 0.05. AGlcSiE represents acylated glucosyl betasitosterol ester. Precursor ions $[M+NH_4]^+$ were detected in positive ion mode following C30-RPLC chromatography. The fatty acids esterified to the sitosterol backbone (molecular species) were identified based on the fragmentation. The lipid components in the table are arranged based on the molecular species composition with the large number before the colon representing total number of carbons, while the numbers after the colon representing the total number of double bonds (eg., 18:3 = 18 carbons with 3 double bonds).

components of root membranes. Conversely, PA is a signaling lipid which can vary significantly in response to stressors in the crop growth environment [66,72,74–76]. In our study, significantly higher levels of PA (up to 24%) was observed in forage soybean roots. This high level is consistently being recorded in other field crops cultivated in our research program [37], indicating high PA may be a common adaptation strategy used by plants to acclimate to the cool temperatures in boreal environments [37,67]. A decrease in soil pH from 6.8 to 6.0 resulted in approximately 80% decrease in forage soybean root PC (Fig. 5). Furthermore, this decrease in PC was concomitant with corresponding increases in PA (24% and 59%), PE (11% and 21%), and AGlcSiE (50% and 185%), respectively (Fig. 5). The major molecular species affected by changes in soil pH included PA 16:0/18:3, PA 18:0/18:2, PA 18:2/18:2, PA 18:3/18:3, PC 18:0/16:0, PC 18:0/18:1, PE 16:1/16:1, PE 18:1/18:1, PE 16:0/18:3, PE 16:1/18:3 (Fig. 6). A decrease in soil acidity from 6.8 to pH 6.0 caused a 47% increase in PA 16:0/18:3 molecular species, whereas a 50% decrease in PA18:0/18:2 and a 40% decrease in PA 18:2/18:2 were observed (Fig. 6). However, when plants were cultivated in acidic soil (5.1), we observed a significant increase in the accumulation of PA18:0/18:2 (by approximately 112%) concomitant with decreased levels of PA18:2/18:2 (46%) and PA18:3/18:3 (45%) compared to when plants were cultivated in the control soil pH of 6.8 (Fig. 6). Conversely, significant increases in PC18:0/16:0 and PC 16:0/20:1 were observed when the soybeans were cultivated in soil containing a pH of 6.0 compared to when cultivated in the control (Fig. 6). In addition to the PA and PC molecular species, the PE molecular species were also remodeled in response to acidic soil (Fig. 6). Plants cultivated at pH of 6.0 had increased accumulation of PE 16:1/16:1 (105% increase), while decreased levels of PE 18:1/18:1 (61%) were observed. In contrast, the levels of PE 18:1/18:1 increased as the soil pH decreased to 5.1 accompanied by decreased levels of PE 16:0/18:3 (40% decrease) and PE 16:1/18:3 (52% decrease) (Fig. 6). These observed changes in root membrane structural (PC, AGlcSiE, and PE) and signaling (PA) lipids appears to be associated with the strategy used by forage soybeans to remodel their root membrane lipids to acclimate to acidic soil when cultivated under cool climatic conditions in boreal ecosystems. Plants are known to regulate their membrane fluidity through lipid remodeling to acclimate to changes in the growth environment [67,69,72,77]. As the soil pH decreased from 6.0 to 5.1, we observed significant shifts in all four major membrane lipid classes as the plants attempted to remodel the root membrane to a composition similar to that observed at control soil pH (Fig. 5). PA acts as a signaling molecule and is the precursor for the synthesis of PE or PC from diacylglycerides (Fig. 6). PE is located on the outer leaflet of the cell

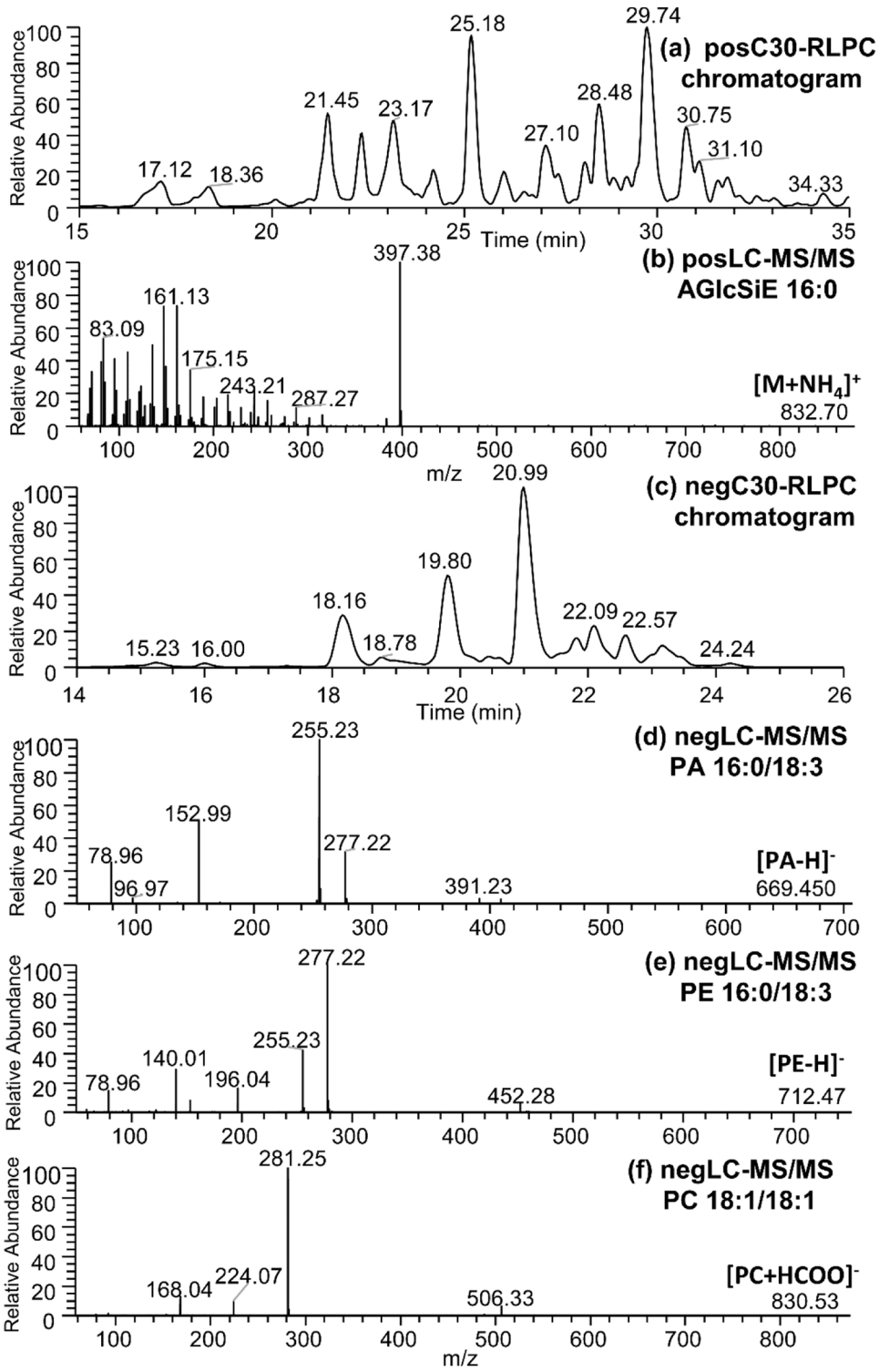

Fig. 2. Chromatograms and mass spectra showing the major membrane lipids and molecular species present in forage soybean roots following cultivation in low acid soil under cool climatic conditions in boreal environments. (**a**) Soybean root membrane lipid analyzed by C30 reverse phase liquid chromatography in positive ion mode and (**b**) *m/z* 832.70 representing AGlcSiE 16:0 $[M + NH_4]^+$ ion (**c**) Chromatogram in negative mode and (**d**) *m/z* 714.50 representing $[PA\ 16:0/18:3\ PA\text{-}H]^-$, and (**e**) *m/z* 712.47 $[PE\text{-}H]^-$ representing PE 16:0/18:3 and (**f**) *m/z* 830.53 representing PC 18:1/18:1 $[PC + HCOO]^-$ precursor ions. AGlcSiE = acylated glucosyl betasitosterol ester, PA = Phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine.

membrane and are ordered crystalline phase lipids that tends to pack closely in the membrane making it more rigid. PC on the other hand, is located on the inner leaflet of the cell membrane and are liquid crystalline phase lipids and do not pack closely in the membrane, making it more fluid or permeable [78–82]. Sterols such as AGlcSiE are thought to be uniformly interspersed within the membrane, where they impart rigidity to the membrane. Thus, the observed alterations suggest reduced fluidity of the membrane may be occurring in response to cultivation in the acidic soil. The native fluidity of the membrane is also the net outcome of the balance between unsaturated and saturated fatty acids present in the membrane [70,71,73].

Low temperature, common in boreal ecosystems, is known to increase fatty acids unsaturation and membrane fluidity [67,69,72,77] consistent with the observations in this study (Table 1–2). This is a well-recognized strategy used by plants to acclimate or adopt to low growth temperatures [70,71,73]. However, forage soybeans appear to be regulating the level to which this increase fluidity and unsaturation occur as a strategy to circumvent the additional stress of acidic soil. The fact that there was generally an increase in molecular species enriched with saturated fatty acids at the expense of molecular species enriched with

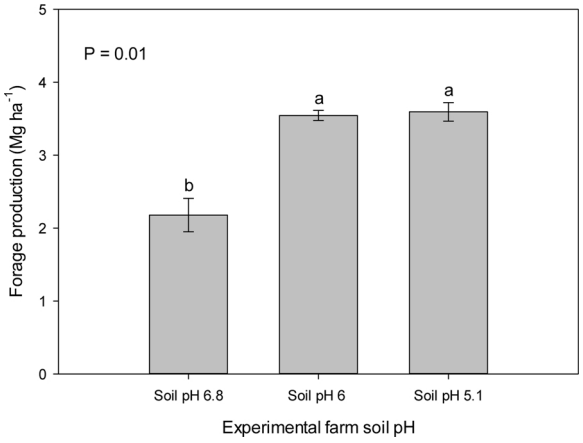

Fig. 3. Effect of soil pH on soybean forage production grown in podzolic soils under cool climatic conditions.

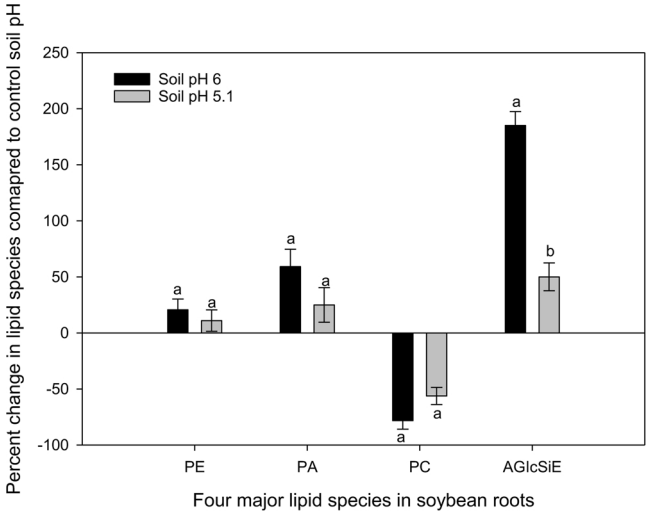

Fig. 5. Effect of soil pH on changes in the major soybean root membrane lipids when cultivated at low soil pH (6.0 and 5.1) compared to control pH (6.8) in podzolic soils under cool climatic conditions in boreal ecosystems.

unsaturated fatty acids in the four major lipid classes remodeled following cultivation in acidic soil (Figs. 5 and 6), further points towards reduced membrane fluidity as a potential strategy used by the forage soybean in circumventing the acidic soil in boreal environments. This is further supported by the observation that forage soybeans cultivated at the acidic soil had similar or superior nutritional composition as the crops cultivated in the control soil pH (Table 3). Following principal component analysis, we observed that 64.5% of total variability was explained by both PC1 and PC2 (Fig. 7ab). We also observed the three soil pH levels clustered in distinct quadrants of the observation or biplots (Fig. 7a) according to forage nutritional quality and the lipids remodeled in the root membrane lipidome. For example, in quadrant-1 (soil pH 6.0) proteins (CP, AP, ACP, ADICP), lipids (PA, SiE, SQDG, AGlcSiE) and relative feeding value of the forage were clustered together (Fig. 7b), and were quantitatively higher at soil pH 6.0 compared to the other two soil pH (Fig. 5). In quadrant-2 (soil pH 5.1), protein (NDICP), lipids (PE), minerals (P, S), forage energies (NEG, NEL, NEM), lignin, TDN, NFC, acid phosphatase activities, SS and forage production were clustered together, and were statistically or quantitatively higher at soil pH 5.1 compared to the other two soil pH (Table 3; Fig. 7). In quadrant-4 (soil pH 6.8), proteins (DP, SP), minerals (Ca, Mg), root membrane lipids (MGDG, DGDG, PI, LPG), ash, ADF, NDFD30 h (Fig. 7b) clustered together and were statistically or quantitatively higher at soil pH 6.8 compared to the other two soil pH (Table 3; Figs. 4 and 5). Root lipids including (PC, LPC, LPE, PG, CL, PS), starch, crude fat, WSC, NDF and K were observed to cluster in Q-3 and contributed more towards soil pH 5.1 and 6.8 compared to soil pH 6.0 (Fig. 7b). Furthermore, the PE, PC, PA and AGlcSiE molecular species and different quality indices also depicted similar patterns for PCA analysis and grouped the three soil pH in different quadrants (Figs. 2–5 DIB [38]). Furthermore, these associations were confirmed through strong correlations between the parameters (lipids, soil pH, forage nutrients) clustered in each quadrant of the PCA observation and biplots (Fig. 7) following analysis by Pearson's correlation (Table 8). For instance, strong positive correlations were observed between soybean root PA and CP (0.75*), and ACP (0.75**) in Q-1; between PE and NDICP (0.74*) in Q-2, and between PC and starch (0.85***) in Q-4 (Table 8). Similarly, significant negative correlation was recorded between PC and CP (-0.69*), NDICP (-0.87***) and forage production (-0.69*) (Table 2, DIB [38]). These findings suggest that the forage proteins appear to be the nutrient most sensitive to the alterations in forage soybean root membrane lipids remodeled following cultivation in acidic soils under cool climatic conditions in boreal ecosystem (Table 8). Environmental stress including acidic soil pH, low temperatures, cadmium and nitrogen have been reported to alter the levels of PC, PE, PA in growing plants including soybean, as well as, enhanced

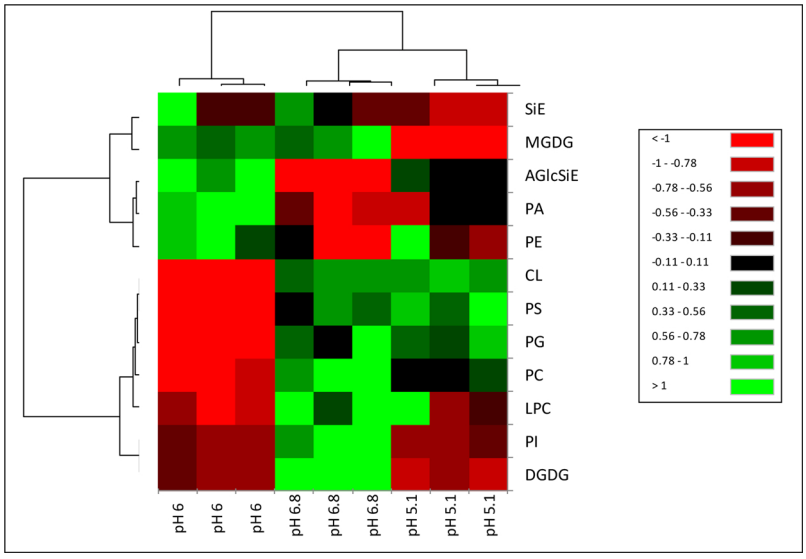

Fig. 4. The heat map showing the variation in soybean root membrane at different soil pH following field cultivation in podzolic soils under cool climatic conditions in boreal ecosystem. Soil pH and root lipids were clustered independently using ascendant hierarchical clustering based on Euclidian distance (Median hierarchical algorithm) at interquartile range of 0.15. Left columns represent the clusters segregating the lipids whereas columns on top segregate the soil pH based on similarities in abundance. Red, black and green colors denote lower, intermediate and higher abundance in the membrane lipid response, respectively. DGDG = digalactosyldiacylglycerol, MGDG = monogalactosyldiacylglycerol, PA = phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PI = phosphatidylinositol, LPC = lysophosphatidylcholine, PS = phosphatidylserine, CL = cardiolipin, SiE = beta sitosterol ester, AGlcSiE = acylated glucosyl betasitosterol ester (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

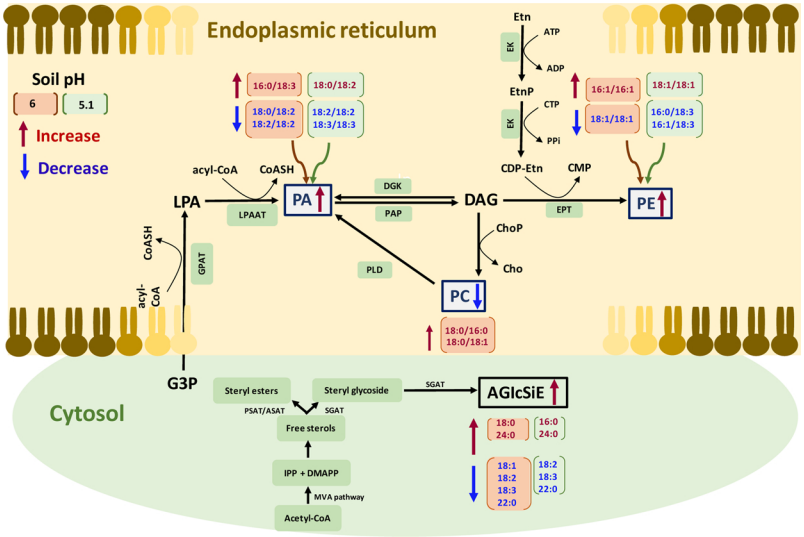

Fig. 6. Lipid synthesis pathways proposed for forage soybean root membrane lipids remodeling following cultivation in acidic pH podzolic soils in cool climate production systems.

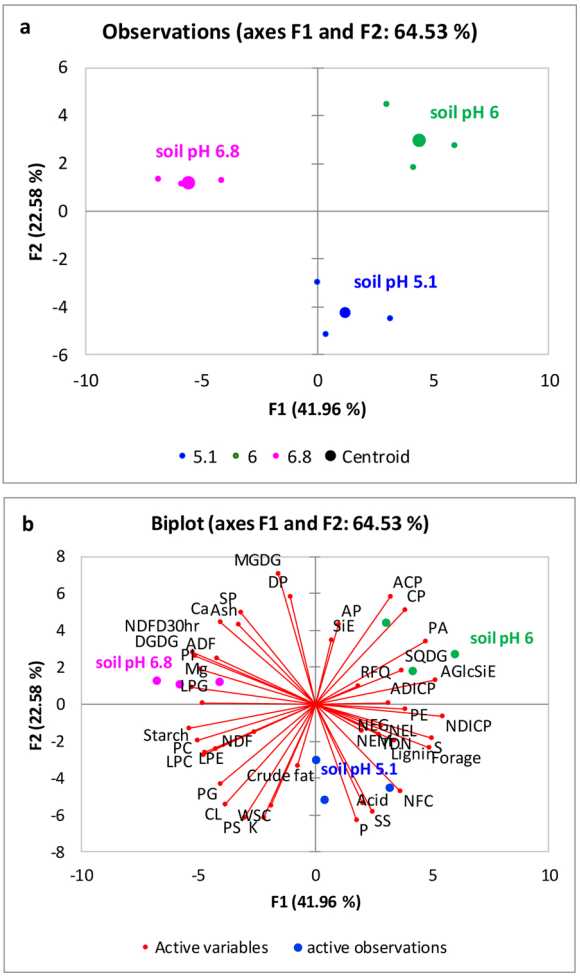

Fig. 7. Principal component analysis showing the relationships between root membrane lipids and forage soybean nutritional quality following cultivation in acidic pH podzolic soil in cool climate production system. **(a)** Observation plot showing segregation of three soil pH based on the centroids on the F1 and F2 axis; and **(b)** Biplot showing relationship between different observations, forage production, quality and membrane lipids at three soil pH.

Table 8
Pearson's correlation coefficient representing the significant relationships between the lipids and forage nutritional quality parameters that clustered in different quadrants of the biplot following cultivation in low soil pH under cool climatic conditions in boreal ecosystem.

Quadrant-1		Quadrant-2		Quadrant-3	
	PA		PE		PC
CP	0.75*	**NDICP**	0.74*	**Starch**	0.85***
ACP	0.75**				

*significant at alpha 0.05, ** at 0.01, and *** at 0.001. Quadrants represent output from Fig. 7.

the unsaturation of membrane lipids consistent with the findings in our study [68,83–86]. Collectively, these findings indicate that the remodeled forage soybean root membrane lipids are important determinants of forage nutritional quality following cultivation under cool climatic conditions in low acidity podzolic soils characteristic of boreal ecosystems.

4. Conclusion

The output from this study demonstrated that forage soybeans remodel their membrane lipids in response to cultivation in acidic soils (agricultural podzols) to produce forage with similar or superior nutritional quality as crops cultivated in near neutral (pH 6.8) under cool climatic conditions in boreal ecosystems. Surprisingly, the forage biomass production was also observed to be higher when cultivated on acidic podzolic soil (5.1 and 6.0) compared to the control pH (6.8). PC, PA, PE and AGlcSiE were the major membrane lipids remodeled in response to cultivation in acidic podzolic soil. PA (C16:0, C18:0, C18:2, C18:3), PC (C18:0), PE (C16:0, 16:1, 18:1), as well as AGlcSiE (C16:0, C18:0, C18:1, C18:2, C18:3, C22:0 and C24:0) enriched molecular species metabolism were enhanced in forage soybean roots as an adaptation strategy to grow and produce forage with superior nutritional quality when cultivated in acidic soils (podzols) in northern climates or boreal ecosystems. Overall, an increase in forage soybean root membrane lipid saturation, and a decrease in unsaturation regulating the fluidity of the membrane appears to be the major response strategy to cultivation in acidic soils. Furthermore, several nutritional components used as indicators of forage nutritional quality was observed to be strongly correlated with the four major root membrane lipids

remodeled during cultivation in agricultural podzolic soils. This work demonstrates, for the first time, how forage soybeans remodeled their root membrane lipids during cultivation in acidic soils, and that the remodeled lipids have strong association with the nutritional quality of the forage produced in cool climate production systems. We hope this work will stimulate further studies in the scientific community to elucidate the mechanisms through which these lipid alterations affect cell membrane phase transitions and membrane function during crop adaption to the changing growth environment in boreal ecosystems.

Author contribution

RT and AN conceptualized the project and designed the experiments. AN performed the field experiment and data collection. RT and AN supervised the experiment. THP, MN, WA, MZ, WA, SSMG, CM and OAA performed biochemical analysis. MN assisted with statistical analysis and wrote first draft of manuscript. RT, LG and MC assisted in writing the manuscript and data interpretation. All authors contributed equally to editing the manuscript.

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