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Variation in frequency of CQA-tested municipal solid waste compost can alter metabolites in vegetables

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ABSTRACT

The use of compost to enhance plant growth and mineral nutrients composition are extensively studied but not much literature information exists on its influence on plant metabolic profiles. A study was performed to assess a 5-year variable frequency of application of Compost Quality Alliance tested municipal solid waste (MSW) compost effect on metabolic profiles of the edible portions of four different vegetable plants. The plants were lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) grown under a sub-humid continental climate. The treatments were annual, biennial and no (control) applications of the MSW compost. Typically, soil fertility highly increased with the annual application of the MSW compost followed by the biennial application but declined in the control plot. The annually applied MSW compost increased total amino acids in the lettuce, carrot, beets, and green beans by *ca.* 323%, 109%, 94% and 18% respectively, compared to the control. Overall, total phospholipids were enhanced by the biennially applied MSW compost. Total organic acids in the lettuce, beets, and green beans were altered by the annual and biennial MSW compost applications by *ca.* 35% and 23%; 6% and 6.4%; and 22% and 65%, respectively compared to the control. A 2-dimension principal component analysis biplot confirmed positive association between the different frequencies of MSW compost application and soil fertility enhancement of plant metabolites. In conclusion, the annual application of MSW compost enhanced amino acids, phospholipids, acylcarnitines, amines and choline but reduced glucose in the lettuce, beets, carrot, and green beans. Further studies to elucidate the mechanisms underpinning such biofortification will be required.

1. Introduction

The integration of recycled organic industrial and municipal solid wastes (MSW) as finished compost into modern farming systems is acknowledged as clean and sustainable technology with a high positive impact on the environment, plant productivity and food safety (Neugart et al., 2018; Vinci et al., 2018; Chaudhary et al., 2020). These organic waste materials are reservoirs of essential plant nutrients and biomolecules such as macro- and micr-nutrients, amino acids, carbohydrates, lipids and mineral nutrients that are vital for plant metabolism. Typically, compost is used alone or in combination with synthetic chemical fertilizers (Hargreaves et al., 2008) to improve soil organic matter, soil structure, water-holding capacity, active microbial community structure and plant growth and harvest quality (Demelash et al., 2014; Chocano et al., 2016; Chaudhary et al., 2020). Macronutrients such as nitrogen, phosphorus and potassium mediate essential cellular pathways involved in the biosynthesis of organic compounds (Bustamante et al., 2019). However, it is not known if MSW compost at varying frequency of application will alter cellular activity and synthesis of plant metabolites.

First generation metabolomics tools have been recently used extensively to provide a broader view of systematic modifications in metabolic processes by analysing numerous targeted and untargeted compounds in plants under varying growing conditions and plant growth stages (Sumner et al., 2015; Zeng et al., 2018). Most of these plant metabolomics studies have focused on providing insight into biotic and abiotic stress tolerance mechanisms (Wi, 2014; Sung et al., 2015; Miyagi et al., 2017; Bueno & Lopes, 2020; Feng et al., 2020). Few studies suggested that compost positively impact metabolic profiles of different plants (Jeffery et al., 2003; Vinci et al., 2018). The application of MSW

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compost enhanced protein and mineral nutrients contents in onion (*Allium cepa*) bulbs (Shaheen et al., 2013). Similarly, Neugart et al. (2018) reported that the metabolic profile of pak choi (*Brassica rapa*) under greenhouse condition was affected by compost and its application rate. Therefore, we presumed that variations in the frequency of MSW compost application can differentially influence plant metabolic profiles. The nutritional and health benefits of most plant metabolites have been proven to prevent various chronic diseases such as cancer, diabetes and cardiovascular diseases (Küllenberg et al., 2012; Larsson et al., 2013; Speer et al., 2019). For instance, dietary lysophosphatidylcholine, which can be found in plants was reported to have beneficial health effect on numerous diseases including cancers and cardiovascular diseases (Küllenberg et al., 2012). As such, we hypothesized that long-term frequent application of MSW compost will potentially biofortify food plants and enhance syntheses of metabolites of human health benefits. Other studies on long-term compost application indicated significant improvement in soil properties and plant productivity (Passoni & Borin, 2009; Chocano et al., 2016; Liu et al., 2019).

Understanding of the metabolic responses of different plant species to the Compost Quality Alliance (CQA)-tested MSW compost application will be beneficial to farmers, nutritionists, consumers and other stakeholders. Additionally, MSW compost application for food production and its benefits to human health and nutrition will gain appreciation. CQA is a voluntary program created by Compost Council of Canada in collaboration with Canadian compost producers to utilize standardized testing methodologies and uniform operating protocols to increase customer confidence in compost selection and utilization. Consequently, this study is important at this critical time when global climate change and indiscriminate use of synthetic chemicals are having a toll on soil quality and the density of nutrients in our foods. These adverse situations have led to increasing food and nutrition insecurity and grave consequences on human health and well-being. Therefore, the present study assessed the effect of a 5-year variable frequency of application of CQA-tested MSW compost on metabolic profiles of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) under a sub-humid continental climatic condition.

2. Materials and methods

2.1. Location and materials

The 5-year on-farm research was carried out in Agaard Farms, Brandon, MB, Canada (Longitude 99° 56′ 59.9892″ W; Latitude 49° 50′ 53.9916″ N; altitude: 409 m above sea-level) between the fall of 2015 and 2019. The climate of Brandon region is described as dominantly cool to moderate cool, Boreal, sub-humid continental with a mean summer (May to September) precipitation of 317.5 mm. Typically, the study location have an annual 1555 mean degree-days and 105 frost-free days (MAFRD, 2010). The soil in Agaard Farms belongs to the Newdale series and characterized by an Orthic Black Chernozem solum on moderately to strongly calcareous, loamy morainal till of limestone, granitic and shale origin. CQA-tested MSW compost was obtained from the waste management facility of the City of Brandon, MB, Canada. Seeds of lettuce, beets, carrot and green beans were purchased from a local greenhouse nursery (The Green Spot, Brandon, MB, Canada).

2.2. Soil analysis

Approximately, 200 g of soil samples from the experimental site were collected in soil collection bags in spring before planting and in fall after planting throughout the five years of the study. Soil samples from the individual treatment plots were randomly collected ($n = 10$) using a portable soil auger from 20-cm depth where most of the root mass of the plants studied were found. Approximately, 300 g of composite samples of the soils were taken for each treatment plot per replication (i.e. 3 treatments × 3 blocks = 9 treatment plots). The soil samples were sent to A&L Canada Laboratory, London, ON for the analyses of soil organic matter, micro- and macro-nutrients, cation exchange capacity and potential hydrogen ion concentration (pH). The 5th-year data on soil analysis is presented in this report.

2.3. Field preparation and planting

The experimental field was ploughed and tilled to a depth of approximately 15 cm in Year 1. The dimension of the experimental field was 80 m × 50 m, which is subdivided divided into three block of dimension 20 m × 10 m. Each block was subdivided into three (control, annual and biennial) to give a total of nine treatments plots each measuring 6 m × 3 m. The separation between blocks was 2 m while the treatment plots were 1 m apart. The annual and biennial plots received CQA-tested municipal solid waste (green bin) compost every year and every other year, respectively. The compost was applied uniformly at a rate of 0.0137 m^3/plot in the fall prior to planting in the spring of the following year. The compost was mixed in the soil during the tilling process. No extra compost nor fertilizer was added throughout the growing season.

Planting was done after last frost-free day in May of each year when the soil temperature is above 10 °C. Seeds of all the four plant species (i.e. carrots, beets, green beans and lettuce) were direct-seeded in single rows in each treatment plot. Plant rows were 75 cm apart between the rows. The carrots were drilled, the beans were 20 cm apart, and the beets and lettuce were 10 cm apart within rows. The plants were irrigated using drip irrigation system when soil was dry and the plants begun to show signs of water-deficit stress. Weeding was done manually using a hand-hoe. No synthetic chemical fertilizer or pesticide were applied to the soil or plants. The edible portions of the plants i.e. young green leaves of the lettuce, roots of the carrots and the beets and green pods of the beans were harvested at edible maturity between 45 and 60 days after sowing the seeds.

2.4. Plant tissue analysis

At the end of the 5th-year, samples of the edible portions of the lettuce, carrots, beets and green beans were collected from 10 plants per block and oven-dried in a 52100-10 Cole-Parmer mechanical convection oven dryer (Cole-Parmer Instrumental Company, Vernon Hills, Ill., USA) at 65 °C for 24 hr until constant weight was achieved. The dried plant tissues were ground using a hammer mill and screened through a 53-μm sieve. The individual grounded plant tissue samples were put in screw cap tubes to an approximate volume of 60 mL (*ca*. 35 g) prior to shipping on ice to Canada's national metabolomics core facility, The Metabolomics Innovation Centre (TMIC), AB, Canada for further sample preparation and duplicate analysis of 120 metabolites comprising amino acids, phospholipids, acylcarnitines, and amines and choline and organic acids.

2.5. DI/LC-MS/MS method

A targeted quantitative metabolomics approach was used to analyze the samples using a combination of direct injection mass spectrometry with a reverse-phase LC-MS/MS custom assay. This custom assay, in combination with an ABSciex 4000 QTrap (Applied Biosystems/MDS Sciex) mass spectrometer, can be used for targeted identification and quantification of up to 143 different endogenous metabolites including amino acids, acylcarnitines, biogenic amines and derivatives, uremic toxins, glycerophospholipids, sphingolipids and sugars (Foroutan et al., 2019, 2020). The method combines the derivatization and extraction of analytes, and the selective mass-spectrometric detection using multiple reaction monitoring (MRM) pairs. Isotope-labeled internal standards and other internal standards were used for metabolite quantification.

The custom assay contained a 96 deep-well plate with a filter plate attached with sealing tape, and reagents and solvents used to prepare the plate assay. First 14 wells were used for one blank, three zero samples, seven standards and three quality control samples. For all metabolites except organic acid, plant extracts were thawed on ice and were vortexed and centrifuged at 13,000*g*. 10 μL of each sample was loaded onto the center of the filter on the upper 96-well plate and dried in a stream of nitrogen. Subsequently, phenyl-isothiocyanate was added for derivatization. After incubation, the filter spots were dried again using an evaporator. Extraction of the metabolites was then achieved by adding 300 μL of extraction solvent. The extracts were obtained by centrifugation into the lower 96-deep well plate, followed by a dilution step with MS running solvent. For organic acid analysis, 150 μL of ice-cold methanol and 10 μL of isotope-labeled internal standard mixture was added to 50 μL of plant extracts for overnight protein precipitation. Then it was centrifuged at 13,000*g* for 20 min. 50 μL of supernatant was loaded into the center of wells of a 96-deep well plate, followed by the addition of 3-nitrophenylhydrazine (NPH) reagent. After incubation for 2 hr, BHT stabilizer and water were added before LC-MS injection. Mass spectrometric analysis was performed on an ABSciex 4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with an Agilent 1260 series ultra-high performance liquid chromatography (UHPLC) system (Agilent Technologies, Palo Alto, CA). The samples were delivered to the mass spectrometer by a LC method followed by a direct injection (DI) method.

2.6. Data analysis

The experiment was arranged in randomized complete block design with three replications. The physical, physical-chemical, and biological characteristics of the compost treated soils and non-compost treated soils were subjected to one-way analysis of variance (ANOVA) using Minitab version 19 (Minitab, Inc., State College, Pennsylvania, USA). When the F-statistic was significant, LSD post hoc test ($P \leq 0.05$) was used to separate the treatment means. Data analysis of the metabolites was done using Analyst 1.6.2. and XLSTAT version 19.1 (Addinsoft, New York, USA). A multivariate statistical analysis of grouped compounds by two- clustering of individual metabolites per group were constructed with Euclidean distance.

3. Results and discussion

3.1. Soil fertility indices

Variation in frequency of application of the CQA-tested municipal solid waste (MSW) compost significantly ($P < 0.05$) altered organic matter content, cation exchange capacity (CEC) and mineral nutrients composition of the soil (Table 1) in the 5th-year of the study. The variations in fertility status were wide range as indicated by the coefficients of variation and ranged from 1.9 to 96.6% depending on the treatment (i.e. compost or soil). Composts are universally acknowledged for their richness in beneficial microbial populations, and humic and non-humic substances that influenced the soil chemical composition as presented in Table 1. We observed that the content of organic matter, the composition of most of the nutrients and estimated nitrogen ratio (ENR) of the MSW compost alone was comparable to those of the soil from the control plot with no MSW compost application. The main reason was that nutrients were immobilized by microbial populations in the MSW compost and was not readily available. Also, the densities of the nutrients were highly increased in the annual and biennial MSW compost applied soils at the end of year five of the study. The cumulative effect of soil factors due to the MSW compost application can also be ascribed to alterations in microbial community and hydrolysing enzymes activities (unpublished). We observed that the application of the MSW compost either annually or biennially significantly ($P < 0.05$) increased soil organic matter content and ENR, but were significantly ($P < 0.05$) decreased in the control plot (Table 1). Compared to the biennial application, the annual MSW compost application non-significantly increased ENR, which is often used to estimate the available N for plant uptake. Overall, essential plant nutrients i.e. N, phosphorus (P) and potassium (K) were outstandingly high in soils that were applied annually with the MSW compost followed by the biennial application. However, soil CEC did not vary among the annual, biennial and the control plots. The slightly high CEC of soil in the control plot can be attributed to the significantly ($P < 0.05$) high contents of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions compared to the MSW compost applied plots.

In contrast, soil reaction (pH), Copper (Cu), manganese (Mn) and magnesium (Mg) contents of the soil were not significantly ($P > 0.05$) altered by the different frequency of MSW compost application. Aluminium (Al) content was similarly low in the cultivated soils irrespective of the MSW compost application frequency or control plots but was 3.73-fold higher in the MSW compost. It is not clear why Al content of the soil was low, especially in the annually applied plots although the MSW compost had high Al content. However, it can be speculated that

Table 1
Fertility status of the MSW compost and five-year treated soils at varying application frequency.

Fertility indices		MSW Compost application				
	MSW Compost alone	AN	BI	NO	Mean	CV (%)
pH	8.10a	7.93ab	7.90b	7.74b	7.92	1.86
OM (%)	2.37b	3.67a	3.17a	2.07b	2.82	26.01
ENR	35.67b	48.67a	43.67a	32.67b	40.17	18.24
CEC (meq/100 g)	15.733b	29.07a	30.6a	33.67a	27.27	29.06
Boron (mg/kg)	0.67b	1.17a	1.17a	0.83b	0.96	26.09
Aluminum (mg/kg)	320.00a	77.00b	61.70b	64.30b	130.75	96.63
Iron (mg/kg)	42.33a	42.00a	39.00a	31.67b	38.75	12.78
Zinc (mg/kg)	3.77b	8.63a	8.23a	4.33b	6.24	40.79
Sulphur (mg/kg)	8.67b	29.67a	28.00a	12.67b	19.75	53.85
Sodium (mg/kg)	20.33c	119.30a	95.00b	22.33c	64.24	78.67
Calcium (mg/kg)	2587.00b	4437.00a	4867.00a	5733.00a	4406	30.12
Potassium (mg/kg)	176.00c	1068.30a	850.00b	154.70c	562.25	83.05
Phosphorus (mg/kg)	68.64b	273.99a	227.71a	90.63b	165.24	61.15
Copper (mg/kg)	1.07a	0.97a	1.00a	0.73a	0.94	15.42
Manganese (mg/kg)	79.94a	106.11a	107.42a	90.48a	95.99	13.73
Magnesium (mg/kg)	273.37a	437.98a	444.79a	499.97a	414.03	23.62

MSW, municipal solid waste; CEC, cation exchange capacity; ENR, estimated nitrogen-release; AN, BI and NO are annual, biennial and no compost application, respectively; rows with different alphabetical letters means significant difference between treatment means at $P \leq 0.05$; and CV is coefficient of variation.

increased plant growth and nutrient uptake might be the cause of low residual soil Al content. Soil content of sodium (Na) and potassium (K) salts were higher in the annual followed by the biennial application plots compared to the control. This was not a surprise since the feedstock for the MSW compost were from household organic wastes that may include foods with added salts. All the other nutrients were similar and significantly ($P < 0.05$) higher in the annual and biennial plots followed by the control, which further confirmed compost enhancement of soil organic matter content and soil fertility status with negligible change in pH (Table 1) due to the good buffering capacity of compost. Previous studies on 10-year field application of compost led to the accumulation of Cu, zinc (Zn) and mercury (Hg) (Avery, 2018) whereas a 14-year study on biosolid application did not alter total soil organic carbon content and heavy metal levels (Börjesson & Kätterer, 2018). Compared to the findings of the present study, the accumulation of these elements in compost and soils will be dependent on chemical composition of feedstock for the compost and the nature and properties of the treated soil.

3.2. Plant metabolites

The metabolites profiles of the tested plants i.e., lettuce cv. Grand Rapids, beets cv. Detroit Supreme, carrot cv. Nantes, and green beans cv. Golden Wax varied with or without MSW compost application determined in the 5th-year of the study. The differences in the compositions of total metabolites suggested differences in plant primary metabolism in response to the variation in the frequency of MSW compost application (Table 2). Seven (7) main groups of essential metabolites of human health benefit were assessed from the edible portions of the tested plants, namely; amino acids, glucose, phospholipids, acylcarnitines, organic acids and amines and choline as explained below.

3.2.1. Amino acids

Annual application of the MSW compost markedly increased total amino acids content in the edible portions of the harvested lettuce, beets, carrot, and green beans by approximately 322.7%, 109.4%, and 18.0%, respectively compared to their counterparts from the control plots (Table 2). For biennial MSW compost application, total amino acids contents were remarkably increased by 276.0% and 128.6% in lettuce and beets, respectively but was reduced by 22.3% and 10.3% in carrot and green beans, respectively compared to the control (Table 2). A similar increase in amino acids content was reported in leaves of pak choi (Neugart et al., 2018) and maize (*Zea mays*) plants (Vinci et al., 2018) in response to compost application. The increase in amino acids by frequent MSW compost application is important for the improvement of biological processes and functions in plants. This is because amino acids are key N reserves for biological carriers involved in internal transport networks between plant organs (Okumoto & Pilot, 2011; Yao et al., 2020).

The remarkable increase in total amino acid in lettuce following annual and biennial MSW compost applications was as a result of high values of proline, valine, threonine, leucine, isoleucine, glutamic acid, phenylalanine, arginine, tyrosine, tryptophan and lysine (Fig. 1A). High values of glycine, alanine, serine, leucine, isoleucine, glutamine, glutamic acid and methionine contents were found in beets harvested from plots applied annually with the MSW compost; while high values of valine, histidine, arginine and tyrosine contents were found in beets harvested from the biennially applied MSW compost plot. Increase in total amino acids in green beans were associated with high values of proline, valine, leucine, isoleucine, aspartic acid, phenylalanine, tryptophan and lysine following annual application of MSW compost while the biennial compost application reduced valine, threonine and arginine contents (Fig. 1A). The increased values of phenylalanine and tyrosine in green beans following biennial MSW compost application might have reduced total amino acids content. This is because phenylalanine and tyrosine are precursors for the biosynthesis of secondary metabolites including phenylpropanoids, hormones and other defence-related compounds (Tzin & Galili, 2010; Mhlongo et al., 2020). Furthermore, studies have shown that stored amino acids are catabolised for energy production in the tricarboxylic acid cycle (Zhu & Galili, 2004; Araújo et al., 2011; Kirma et al., 2012). This biochemical process leads to the production of organic acids and consequent reduction in total amino acids (Mazelis, 1980; Lopez-Bucio et al., 2000; Hildebrandt et al., 2015), as found in the biennial MSW compost treated green beans (Fig. 1A).

Table 2
Metabolites profiles of edible portions of vegetable plants applied with varying frequency of MSW compost for five years.

MSW compost application	Metabolites (mg/100 g of edible portion)						
	Amino acids	Glucose	Organic acids	Phospholipids	Acylcarnitine	Amines	Choline
Lettuce							
AN	132.58	786.21	39.45	1.16	0.32	1.74	15.91
BI	118.22	980.67	35.95	0.87	0.33	1.61	17.50
NO	31.44	1346.21	29.20	1.08	0.18	0.54	9.19
Mean	94.08	1037.70	34.87	1.03	0.28	1.30	14.20
CV (%)	58.17	27.40	14.95	14.64	30.72	50.97	31.07
Carrot							
AN	34.45	2524.11	25.50	1.12	0.15	0.83	5.53
BI	13.78	3464.90	14.86	1.48	0.18	0.50	4.22
NO	17.73	2570.81	37.68	1.15	0.16	0.53	3.56
Mean	21.98	2853.27	26.01	1.25	0.16	0.62	4.44
CV (%)	49.91	18.58	43.89	16.07	7.36	29.64	22.59
Green beans							
AN	153.73	1324.93	26.51	1.92	0.29	1.73	10.38
BI	116.00	736.42	35.65	1.19	0.23	1.17	7.94
NO	130.33	1007.47	21.67	1.12	0.26	0.99	7.88
Mean	133.35	1022.94	27.94	1.41	0.26	1.30	8.73
CV (%)	14.28	28.80	25.40	31.61	11.27	29.75	16.33
Beets							
AN	237.04	302.75	52.73	0.92	0.18	0.44	2.96
BI	258.78	209.79	52.90	1.14	0.22	0.70	3.69
NO	113.20	316.29	49.71	1.04	0.16	0.31	2.99
Mean	203.01	276.27	51.78	1.03	0.19	0.49	3.21
CV (%)	28.68	20.98	3.47	10.44	14.76	41.30	12.84

MSW, municipal solid waste; AN, BI and NO are annual, biennial and no compost application, respectively; CV, coefficient of variation.

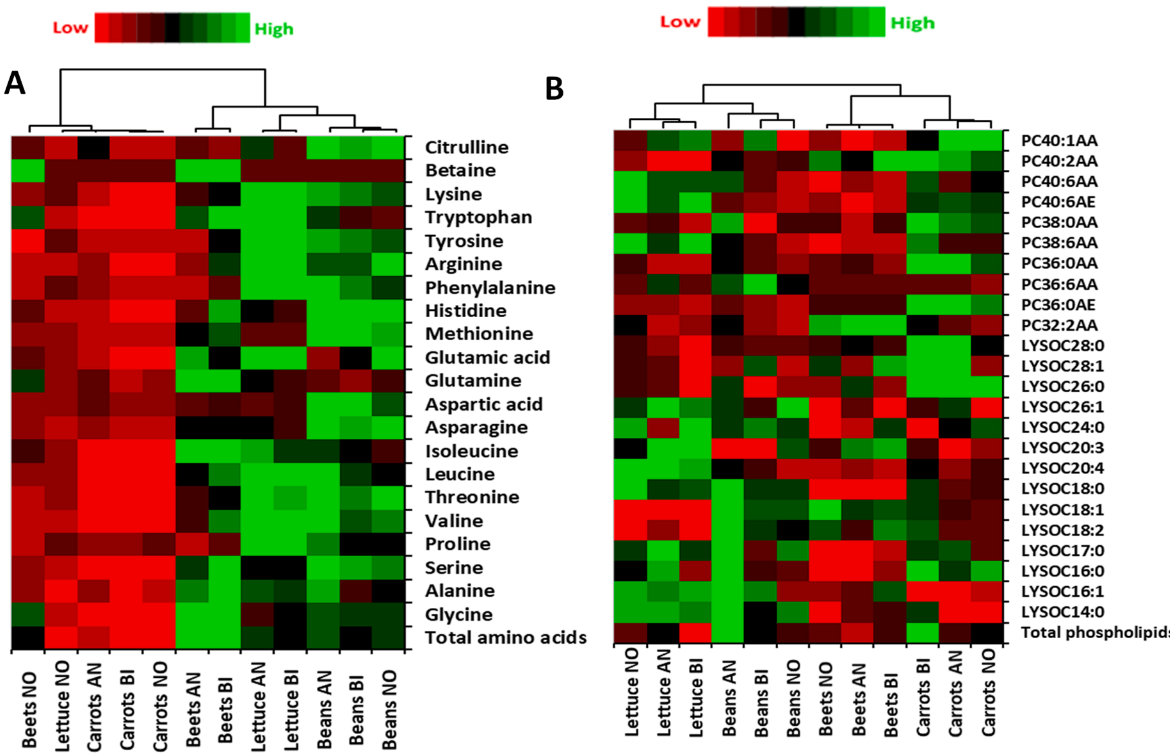

Fig. 1. Heat map of metabolites profiles for total and specific amino acids (A) and phospholipids (B) of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) under a sub-humid continental climatic condition with hierarchical clustering showing patterns of metabolic changes as influenced by variation in frequency (annual, biennial and control) of compost quality assurance tested municipal solid waste compost application. Metabolite concentrations in each block compartment are normalized across all data for one individual compound such that similar colour intensities between compounds can represent widely differing concentrations. Red colour represents lower concentration and green colour represents higher concentration of a particular metabolite as shown in the legend. Lyso, lysophosphatidylcholine; PC, phosphatidylcholine; AN, BI, NO are annual, biennial and no (control) compost application, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The change in total amino acids content in carrot seemed marginal. There was a slight increase in total amino acids in carrot applied annually with the MSW compost and moderate reduction in carrot that was applied biennially (Fig. 1A). This finding conforms to the report by Kaack et al. (2001). The reduction in amino acids content in carrot following biennial MSW compost application can be ascribed to changes in carbon partitioning as demonstrated by the increase in glucose content compared to the annual application of MSW compost (Table 2). Therefore, frequent application of MSW compost highly enhanced total amino acids in lettuce, beets and carrot but not green beans under the conditions of this study. It is well known that compost improves soil fertility by enriching soil microbial communities, amino acids and soil nutrients (Demelash et al., 2014). These soil enrichment properties might have contributed to the high amino acids content in the plants as previously explained by Bustamante et al. (2019).

3.2.2. Glucose

Both annual and biennial MSW compost applications reduced glucose content in the lettuce by approximately 41.6% and 27.2%, respectively; and by approximately 4.3% and 33.7% respectively in beets (Table 2). High soil N modulate the uptake and assimilation of N through enzymatic regulation, which can reduce glucose accumulation while amino acids are increased in plants (Ertani et al., 2013; Pratelli & Pilot, 2014). This can explain the high amino acids contents in the lettuce, beets and carrot (Fig. 1A). Similarly, a study on carbon partitioning in selected crops including beets revealed that low P levels enhanced the synthesis of carbon compounds including glucose as a result of increased activities of starch metabolic enzymes (Rao & Terry, 1995; Hammond & White, 2011). Besides, exogenous P application reduced sugar content (Rao & Terry, 1995), which can also explain the low glucose contents in those plants harvested from plots with high soil P content due to annual or biennial application with MSW compost. The annual MSW compost application increased glucose content in green beans by approximately 31.5% but the glucose content was reduced by approximately 1.8% in carrot when compared to their control counterparts (Table 2). Similarly, the biennial application of MSW compost increased glucose content by 38% in carrot but this was reduced by 26.9% in green beans. The results suggested that as carrot plants accumulates higher photosynthates for growth, the increased partitioning of glucose into the root (i.e. storage organ) reduce amino acids accumulation.

3.2.3. Phospholipids

Total phospholipids content in the lettuce was increased slightly by 7.4% due to the annual application of the MSW compost (Table 2). Specifically, the increased total phospholipids content in lettuce was due to increased values of lysophosphatidylcholine (Lyso) C16:0, LysoC17:0, LysoC20:3, LysoC26:1, phosphatidylcholine (PC) 36:6AA and PC40:1AA (Fig. 1B). However, the biennial MSW compost application reduced total phospholipids content in lettuce by 19.4% as a result of reduced values of LysoC16:0, LysoC18:0, LysoC20:4 and PC40:6AA compared to the control. For the green beans, the annual and biennial MSW compost applications increased total phospholipids content by 71.4% and 6.3%, respectively compared to the control (Table 2). The annual application of MSW compost increased individual phospholipids more than the biennial application. The increment of phospholipids by the annual MSW compost application can be ascribed to increased values of seven individual phospholipids i.e. LysoC14:0, LysoC16:0, LysoC18:2, LysoC18:0, LysoC20:4, PC38:0AA and PC40:6AA in the green beans

(Fig. 1B). On the other hand, the enhancement of total phospholipids in green beans by the biennial MSW compost application can be ascribed to increased values of four specific phospholipids i.e. LysoC16:1, LysoC28:1, LysoC36:6AA and PC40:1AA (Fig. 1B). The least phospholipids were found in plants harvested from the control plots without compost treatment. High values of LysoC16:1, LysoC18:1, LysoC20:3, LysoC24:0 and LysoC28:1 following biennial application of the MSW compost increased total phospholipids content in beets by 9.6% compared to the control (Fig. 1B; Table 2). On the contrary, total phospholipids content was reduced by 11.5% when MSW compost was applied annually compared to the control (Table 2) due to low values of LysoC18:2, LysoC18:1, LysoC28:1 and PC40:2AA (Fig. 1B). Similarly, the annual MSW compost application reduced total phospholipids by 2.7% in carrot as a result of low values of LysoC16:0 and LysoC24:0; while the biennial MSW compost application enhanced total phospholipids content in carrot by 28.7% due to high values of LysoC14:0, LysoC17:0, LysoC18:2, LysoC18:1, LysoC18:0, LysoC28:1, LysoC28:0 and PC38:6AA.

Overall, total phospholipids in all the four different plant species i.e., lettuce, beets, carrot, and green beanswere enhanced by the biennial application of MSW compost. Phospholipids are found in most plant cell membranes and are known to mediate several biochemical and cellular processes including defence, hormone, gravitropism and cell elongation through various interactions with membrane proteins (Ryu, 2004). Therefore, based on the results we expect that these biochemical and cellular processes will be higher in plants that were grown in soils that received the biennial MSW compost treatment followed by the annual and then the control. It was obvious from the heat map in Fig. 1B that the composition of the individual phospholipids in the edible portions of the different plant species were altered by the differences in MSW compost treatment. According to Lei Liu et al. (2014), the composition of phospholipids is highly influenced by variations in plant species, genotype and environmental conditions. In conformity with the present study, Vinci et al. (2018) reported higher phospholipids (i.e. phosphatidylcholine and its intermediates) accumulation in maize leaves upon application of combined compost and *Trichoderma* amendment. Therefore, the significance of key phospholipids in plant growth and development will have to be explored further.

For instance, it is known that lysophosphatidylcholine is crucial for starch biosynthesis (Liu et al., 2013), and it was also suggested as an intracytoplasmic messenger that connects stress-responding enzymic activation and vacuolar proton flux for defence activation (Viehweger et al., 2002). Therefore, the accumulation of these phospholipids may suggest increased protection of membrane integrity against biotic and abiotic stress damage in compost applied plants, especially the biennial treatments. Similarly, other studies revealed that elicitors enhance lysophosphatidylcholine synthesis in plants through transient increase in phospholipase activity (Chandra et al., 1996; Scherer et al., 2002; Viehweger et al., 2002). Typically, compost contains numerous microorganisms that may produce elicitors that seemed to have increased the accumulation of lysophosphatidylcholine in the MSW compost treated plants in the present study. However, further investigation is required to clearly elucidate the effect of short- to long-term application of MSW compost on phospholipids activities. Additionally, dietary lysophosphatidylcholine have beneficial health effect on many human diseases including cancers and cardiovascular diseases (Küllenberg et al., 2012). It is therefore, a good news that we can enhance such important phospholipid of immense human health benefit using low-cost and sustainable organic amendment.

3.2.4. Acylcarnitine

Like the other metabolites, total acylcarnitine contents in the edible portions of lettuce and beets were increased by MSW compost application. Annual application of MSW compost increased total acylcarnitine in lettuce and beets by 77.8% and 83.3%, respectively compared to the control (Table 2). Increments due to the biennial MSW compost application were 12.5% and 37.5%, respectively compared to the control. The remarkable increase in total acylcarnitine in lettuce were influenced by the high values of C0, C3:1, C3OH, C5:1, C6:1, C5OH, C5:1DC, C5DC, C8, C9, C10:1, C10, C16:2 and C16 contents following both annual and biennial MSW compost applications and distinctively, high values of C7DC, C10:2, C12DC, C16:1OH, C18 for the annual and C4:1, C14:1, C18:2 for the biennial compost applications (Fig. 2A). Similarly, high values of C3:1, C5, C5:1DC, C8, C10:2, C10, C14:2, C12DC, C18:1, C18:0 and C18:1OH were recorded for beets harvested from plot applied biennially with the MSW compost while high values of C16:2 and C12:2 were recorded for beets harvested from annually applied plot. It was reported that maize plant exposed to cold stress and exogenously applied carnitine accumulated acylcarnitine in their leaves, which negatively imparted phospholipids accumulation (Turk et al., 2019). Therefore, the increase in acylcarnitine contents in the lettuce and beets in this study could be an indication of a reduced total phospholipids content (Fig. 1B) under annual and biennial MSW compost applications. Besides, the increase in acylcarnitine in edible portions of the plants harvested from the annually or the biennially applied MSW compost plots can be attributed to stimulation of carnitine acyltransferase activities as reported by (Turk et al., 2019). Nevertheless, further studies are needed to examine this effect.

The annual application of the MSW compost application had similar effect on total acylcarnitine in carrot while the biennial application slightly increased it by 12.5% compared to the control (Table 2). The low carrot total acylcarnitine content can be ascribed to the low values of C14:2OH, C16:1, C16:2OH and C18:2 for both MSW compost application frequencies in addition to low values of C4:1, C3:OH, C5DC, C14:1, C14:OH, C16OH due to the annual treatments alone (Fig. 2A). Nonetheless, the annual MSW compost application increased total acylcarnitine content in green beans by 11.5% while biennial application reduced total acylcarnitine content by 11.5% compared to the control. Specifically, the increased total acylcarnitine in green beans harvested from plot applied with the annual MSW compost can be ascribed to the high values of C3:1, C4, C5:1, C6:1, C5DC, C9 and C18:1OH compared to the biennial application with low values of C3OH, C5OH, C5DC, C10:2, 10, C14:2, C12DC, C16:2, C16 and C16:OH (Fig. 2A). According to Nguyen et al. (2016), the accumulation of acylcarnitine in canola (*Brassica napus*) occurred concurrently with an increase in oil yield during morphogenetic and postembryonic phases of seed development, and was implicated in the activation of genes in the lipid biosynthetic pathway during seed germination. Consequently, it is possible that the variations in the frequency of MSW compost application modulated acylcarnitine and phospholipids syntheses in the different vegetable plants. Thus, the modulation impacted primary and secondary metabolic pathways of lipids but will need to be investigated further.

3.2.5. Organic acids

The MSW compost application differentially altered total organic acids content in the edible portions of the lettuce, green beans and beets by 35.1%, 22.3% and 6.1% for the annual application; and 23.1%, 64.5% and 6.4% for the biennial application, respectively compared to the control (Table 2). The increase in lettuce total organic acid content was influenced by high values of beta-hydroxybutyric acid and isobutyric acid for both frequencies of MSW compost applications. Additionally, the annual MSW compost application also increased the value of butyric acid content of lettuce (Fig. 2B). Likewise, the increase in total organic acids in green beans grown in plots annually applied with the MSW compost was influenced by high values of pyruvic acid, hippuric acid, lactic acid and beta-hydroxybutyric acid while high values of citric acid, pyruvic acid and hippuric acid led to a high total organic acids content in green beans harvested from plots that were biennially applied with MSW compost (Fig. 2B). The accumulation of organic acids in plants have been reported to be induced by soil nutrients availability and environmental stress conditions (Ciereszko et al., 1996; Lopez-Bucio

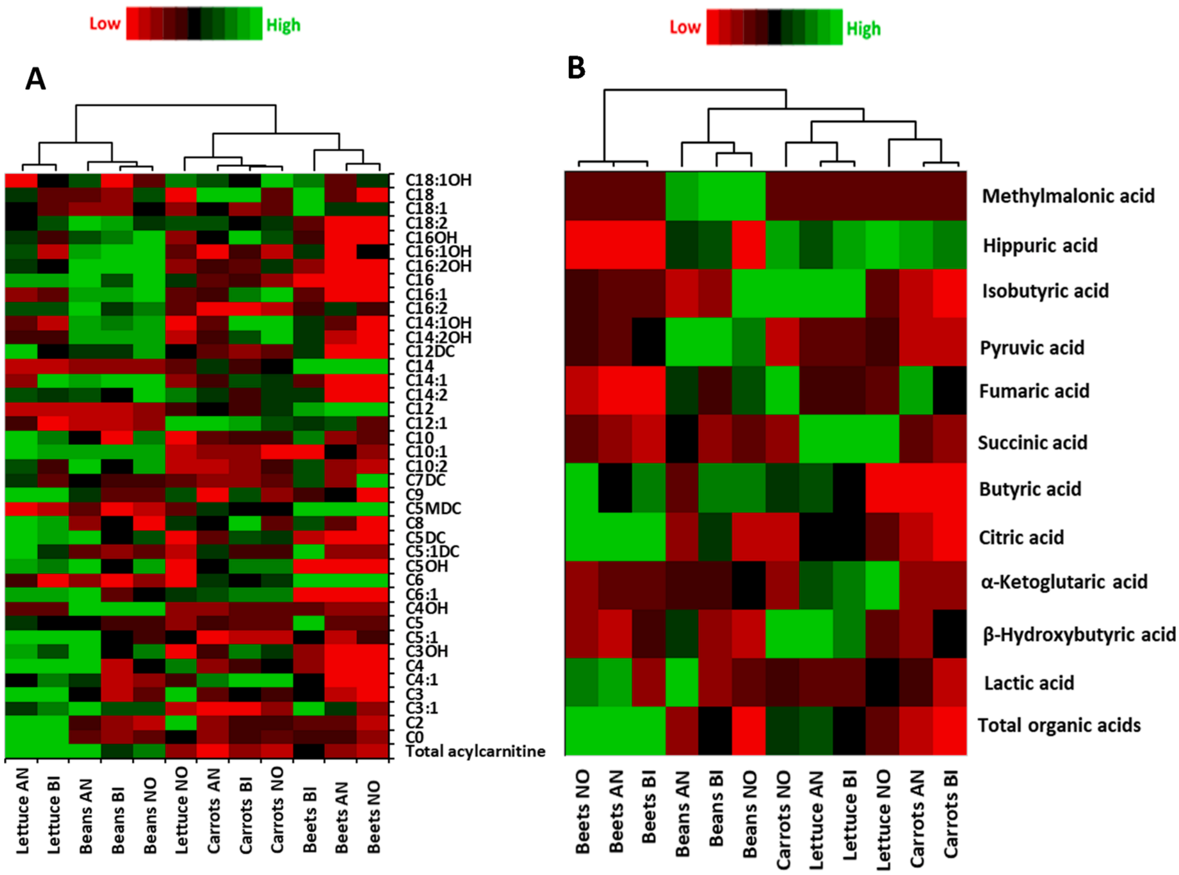

Fig. 2. Heat map of metabolites profiles for total and specific acylcarnitine (A) and organic acids (B) of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) under a sub-humid continental climatic condition with hierarchical clustering showing patterns of metabolic changes as influenced by variation in frequency (annual, biennial and control) of compost quality assurance tested municipal solid waste compost application. Metabolite concentrations in each block compartment are normalized across all data for one individual compound such that similar colour intensities between compounds can represent widely differing concentrations. Red colour represents lower concentration and green colour represents higher concentration of a particular metabolite as shown in the legend. Lyso, lysophosphatidylcholine; PC, phosphatidylcholine; AN, BI, NO are annual, biennial and no (control) compost application, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2000). For instance, it was reported that low P in soil increased the transportation of assimilated carbon from bean plant root to shoot, which led to increased sugar partitioning and a resultant reduction in amino acids and organic acids contents (Ciereszko et al., 1996). This observation suggested that high P level can be associated with increased total organic acids content but reduced glucose content, which can explain the finding in this study.

On the other hand, both the annual and biennial MSW compost applications reduced total organic acids content in carrot by 32.3% and 60.6%, respectively compared to the control (Table 2). This marked reduction can be ascribed to low the values of beta-hydroxybutyric acid, butyric acid, fumaric acid and isobutyric acid contents (Fig. 2B). Among these organic acids, butyric acid and isobutyric acid are low molecular weight carboxylic acids or volatile short-chain fatty acids. They were identified as by-products of fermentative microbial decomposition of organic matter present in compost (Himanen et al., 2006). As a result, butyric and isobutyric acids might be present in the MSW compost used in this study but were not measured, and their mechanism of transport into the plants from the soil or otherwise is unknown. Moreover, the high organic matter content of the soil as a result of the MSW compost application irrespective of the application frequency (Table 1) suggested high microbial activities leading to high volatile fatty acids production in the soil. Nevertheless, the increased accumulation of butyric acid and isobutyric acid in the edible portions of the lettuce (leaves), beets (roots) and green beans (pods) may be attributed to their possible transport across root cells into edible organs as reported for barley (*Hordeum vulgare*) by Lee (1977). However, the reduction of these volatile compounds in the roots of carrot may be due to loss of organic acids as root exudates and/or through other metabolic processes, which must be investigated further. Functionally, butyric and isobutyric acids are energy-rich, which can act as food reserves in seeds (Ulbright, 1981) and may be involved in abiotic stress resistance in plants (Li et al., 2017).

3.2.6. *Amines*

Total amines were enhanced in the edible portions of the tested plants by the MSW compost irrespective of the frequency of application compared to the control. The annually applied MSW compost increased total amines contents of the lettuce, beets, carrot, and green beansby 222.2%, 41.9%, 56.6%, and 74.7%, respectively compared to their individual control treatments (Table 2). The biennial MSW compost application increased total amines by 198.1%, 125.8%, and 18.2% in the lettuce, beets, and green beans respectively but it was reduced by 5.7% in carrot compared to their control counterparts. Noticeably, the composition of total amines as influenced by the different frequency of MSW compost application varied amongst the different plant species. Specifically, the increase in total amines content in the lettuce was due to high values of putrescine, *trans*-hydroxyproline, alpha-aminoadipic acid, methionine-sulfoxide and spermidine following annual or biennial application of MSW compost (Fig. 3). It is worth noting that the spermidine content of the lettuce from the annually applied MSW

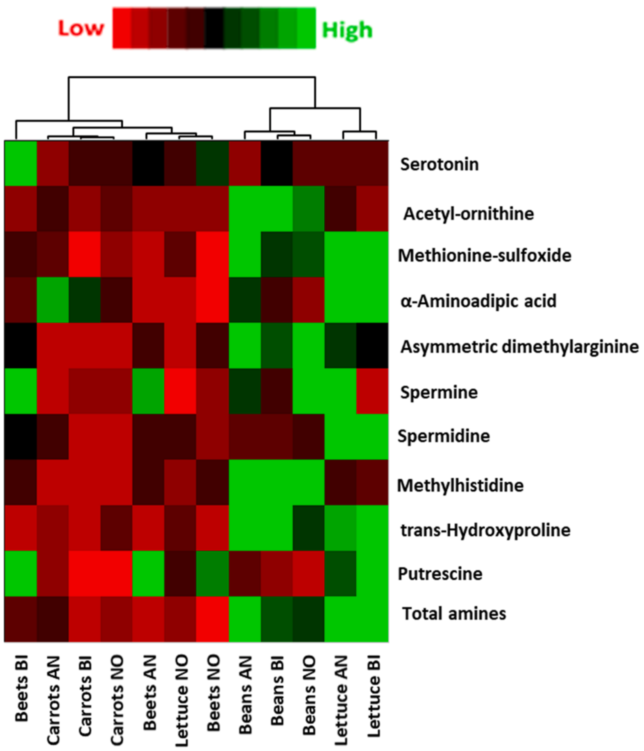

Fig. 3. Heat map of metabolites profile for total and specific amines and choline of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) with hierarchical clustering showing patterns of metabolic changes as influenced by variation in frequency (annual, biennial and control) of compost quality assurance tested municipal solid waste compost application. Metabolite concentrations in each block compartment are normalized across all data for one individual compound such that similar colour intensities between compounds can represent widely differing concentrations. Red colour represents lower concentration and green colour represents higher concentration of a particular metabolite as shown in the legend. Lyso, lysophosphatidylcholine; PC, phosphatidylcholine; AN, BI, NO are annual, biennial and no (control) compost application, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compost plot was higher than that of the biennial treatment. Similarly, increased total amine contents in the green beans were influenced by high values of alpha-aminoadipic acid and methionine-sulfoxide following annual MSW compost application in addition to high values of *trans*-hydroxyproline and acetyl-ornithine, which were increased by both the annual and biennial MSW compost applications. Notably, total amine contents in beets were increased by high values of putrescine and spermine following annual and biennial compost treatments. However, increased total amine contents in carrot was influenced by the high value of spermidine and alpha-aminoadipic acid content following annual MSW compost application (Fig. 3).

Putrescine, spermine and spermidine are derived from amino acids such as arginine and ornithine and have been identified to play a major role in plant stress responses (Gill & Tuteja, 2010; Barnaby et al., 2015; Seifi & Shelp, 2019; Islam et al., 2020). An increase in putrescine was previously reported in deoxynivalenol treated wheat (Warth et al., 2015) and carbon dioxide treated potato leaves (Barnaby et al., 2015) in response to biotic and abiotic stressors, respectively. On the other hand, the slight reduction in total amines due to biennial MSW compost application can be attributed to low value of spermidine content in the edible portions of the tested plants. Similar studies demonstrated biological function of polyamines (putrescine, spermidine and spermine) in abiotic stress mitigation possibly *via* osmotic adjustment and scavenging of reactive oxygen species under multiple abiotic stress responses (Alcázar et al., 2011; Alet et al., 2011; Barnaby et al., 2015; Islam et al., 2020). The dominantly cool to moderate cool, Boreal, sub-humid continental conditions of Brandon where the study was performed can be stressful to plants and perhaps, the syntheses of these biogenic compounds might have contributed to stress tolerance in the MSW compost treated plants. Polyamines have multi-array of roles in human health and nutrition, which includes cellular metabolism, biosynthesis of protein, ribonucleic acid and deoxyribonucleic acid (Bardócz, 1995). As such, their augmentations, particularly in the lettuce and the green beans by the application of MSW compost must be further explored.

3.2.7. Choline

Choline is known to play key roles in plant growth and development (Ahmad et al., 2008). Oxidation of choline produces glycinebetaine, an osmolyte that accumulates in plants to mitigate various abiotic stress conditions (Sakamoto & Murata, 2000; Salama et al., 2011). Likewise, the MSW compost increased choline contents in the edible portions of the plants compared to the control (Table 2). The annually applied MSW compost increased choline contents of lettuce, carrot and green beans by 73.1%, 55.3% and 31.7% respectively; while the biennial treatment increased total choline contents by 90.4%, 0.8%, and 18.5% respectively compared to their individual control treatments. For beets, annual MSW compost application reduced choline content by small amount of approximately 1% compared to the control treatments. In contrast, biennial MSW compost treatment enhanced choline content by 23.4% compared to the control. In lipid signalling, choline act as a precursor for phosphatidylcholine, which plays a fundamental role in ion regulation and membrane stability (Salama & Mansour, 2015). According to Zhang et al. (2010), the beneficial soil microbe *Bacillus subtilis* can enhance choline and glycinebetaine accumulation with improved tolerance to osmotic stress in *Arabidopsis*. Other studies revealed a strongly positive association between choline build-up versus drought, salt and oxidative stress responses (Ahmad et al., 2008)] as well as interaction with various *Rhizobia* strains (Gou et al., 2015). But the question is: could it be that soil microbes were stimulated by the MSW compost application to increase choline uptake and/or accumulation by the plants? If so, then the high accumulation of choline in lettuce (i.e. biennial > annual by *ca.* 18%) and green beans (i.e. annual > biennial by *ca.* 31%) followed by carrot (i.e. annual > biennial by *ca.* 24%) and then beets (i.e. biennial > annual by *ca.* 20%) may thus, be attributed to plant root-microbial interaction that may lead to plant protection against biotic and abiotic stresses. The differences in plant response to annual versus biennial application can be attributed to genotypic characteristics with respect to application frequency, which needs further investigation.

3.2.8. Association between nutrients and metabolites

The mean glucose contents of the edible portions of the four tested vegetable plants had a significant and strong negative association with the mean total organic acids ($r = -0.74$, $P = 0.006$; Table 3) and total amino acids ($r = -0.81$, $P = 0.001$). This relationship can explain the high total organic acids and high total amino acids in beets and lettuce with reduced glucose contents (Table 2). Similarly, total amino acids had a significantly moderate positive correlation with total organic acids ($r = 0.67$, $P = 0.016$), which is in contrast with the total phospholipids content with a moderately negative association with total organic acids ($r = -0.50$, $P > 0.05$). Total acylcarnitine also showed a significantly strong positive correlation with choline ($r = 0.88$, $P = 0.000$), total amines ($r = 0.88$, $P = 0.000$) and slight to no relationship with the other metabolites (Table 3).

To further assess the extent of association amongst the different metabolites and the different plant species, a two-dimension (2-D) principal component analysis (PCA) biplot was used (Fig. 4). The 2-D PCA biplot showed projections of the variables in the factors space (i.e. F1 and F2) and explained 80% of the total variations in the dataset. The responses of the different plant species to the experimental

Table 3
Pearson correlation coefficients (r) amongst metabolic compounds in the four different plants (lettuce, beets, carrot and green beans) and their significance at P ≤ 0.05.

	Amino acids	Glucose	Phospholipids	Acylcarnitine	Organic acids	Amines
Glucose	r = −0.808 P = 0.001					
Phospholipids	r = −0.106 P = 0.742	r = 0.359 P = 0.252				
Acylcarnitine	r = 0.339 P = 0.282	r = −0.353 P = 0.261	r = 0.143 P = 0.658			
Organic acids	r = 0.673 P = 0.016	r = −0.739 P = 0.006	r = −0.489 P = 0.107	r = −0.070 P = 0.828		
Amines	r = 0.127 P = 0.695	r = −0.280 P = 0.378	r = 0.006 P = 0.986	r = 0.883 P = 0.000	r = −0.059 P = 0.855	
Choline	r = −0.014 P = 0.966	r = −0.163 P = 0.614	r = −0.010 P = 0.975	r = 0.876 P = 0.000	r = −0.200 P = 0.533	r = 0.976 P = 0.000

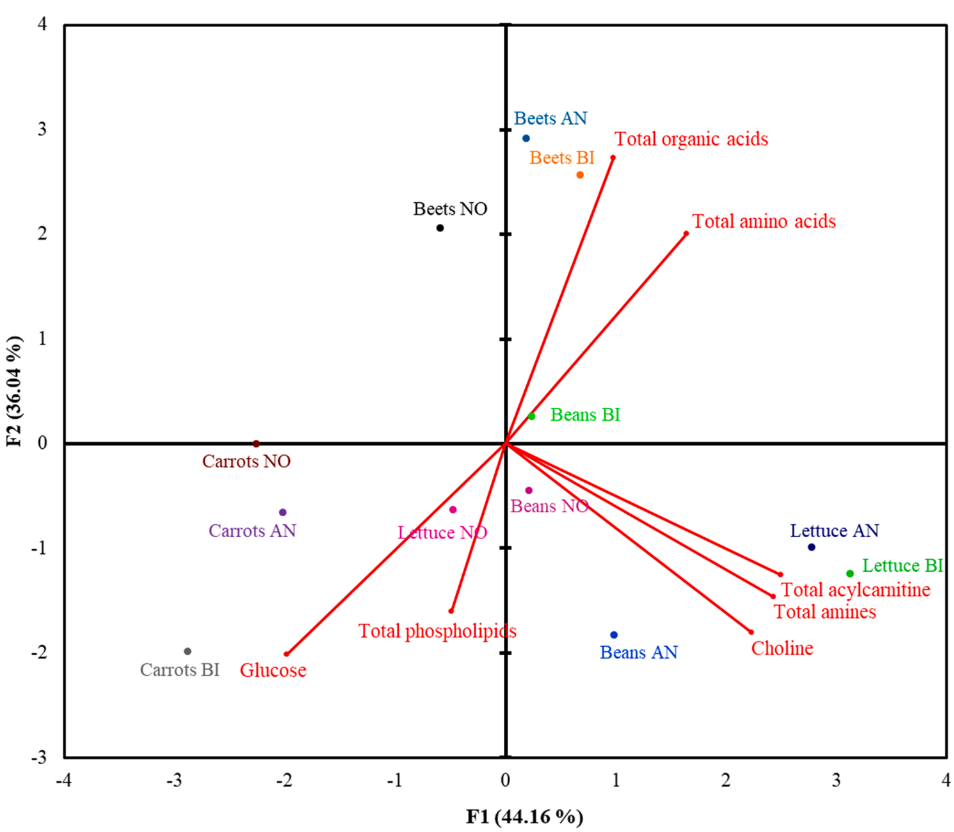

Fig. 4. A two-dimensional principal component (2-D PCA) analysis biplot showing relationships amongst the explanatory variables (metabolites) total acylcarnitine, phospholipids, glucose, organic acids amino acids, amines and choline of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) as influenced by variation in frequency (annual, biennial and control) of compost quality assurance tested municipal solid waste compost application. AN, BI, NO are annual, biennial and no (control) compost application, respectively. Projection of the variables in the 2-D factor space (F1 and F2) explained a total of 80.2% of the variations in the dataset. Variables that are closely located are not different compared to variables located at a distant within a quadrant or between quadrants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

treatments differed, hence the separation of their locations on the 2-D PCA biplot. However, each of the plant species formed a cluster but the individual dataset was separated based on the variations in their responses except the no compost treated lettuce that is located in quadrant 2 with the carrot (Fig. 4). The variation in the plant metabolites due to differences in treatments was clearly represented on the PCA plot. Firstly, beets harvested from the annually applied MSW compost plot had higher organic acids and amino acids contents followed by the biennial then the control but low glucose, phospholipids, acylcarnitine, amines and choline contents. The clustering also suggested high content of inherent amino acids and organic acids contents in beets irrespective of treatments and as reported in previous studies (Kapadia & Rao, 2012; Maheshwari et al., 2013). This can be attributed to the modulation of N uptake and assimilation by regulating enzymes involved in N metabolism (Ertani et al., 2013; Pratelli & Pilot, 2014). Likewise, the MSW compost treated and control carrots showed high contents of glucose and phospholipids but contents of low amino acids, organic acids, acylcarnitine, amines and choline (Fig. 4). Additionally, lettuce grown in plots annually or biennially applied with MSW compost can be associated with high contents of acylcarnitine, amines and choline, moderately high contents of amino acids and organic acids, and low content of glucose and phospholipids. Furthermore, the annually and biennially applied MSW compost-treated beans, control beans and control lettuce showed high phospholipids, and moderate glucose, acylcarnitine, amines and choline contents with low amino acids and organic acids contents.

In Fig. 5, the 2-D PCA biplot further delineates the association between soil fertility status in response to MSW compost application and plant metabolites. The impact of the treatments varied and located differently on the 2-D PCA biplot, which explained 100% of the variations in the dataset. It was confirmed that the application of the MSW compost can be positively associated with soil fertility enhancement. On the contrary, high soil CEC, Ca, Mg and pH were associated with the control treatment (i.e. no application of MSW compost) as explained earlier. The rise in soil pH can be associated with reductions in soil Mg, Ca and CEC. Among the identified plant metabolites, total acylcarnitine,

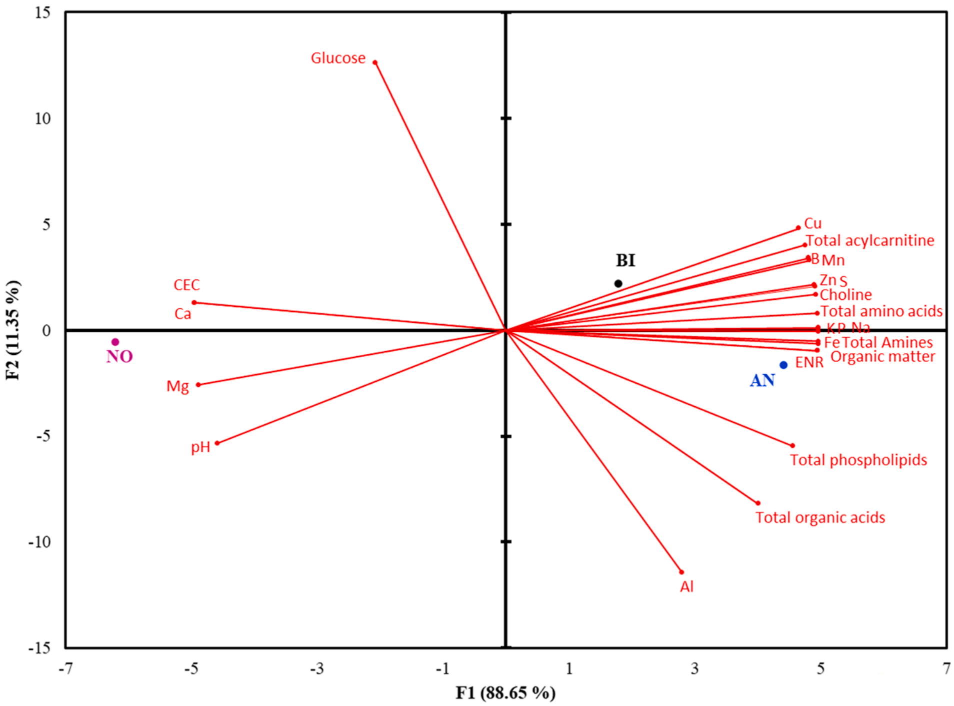

Fig. 5. A two-dimensional principal component (2-D PCA) analysis biplot showing relationships amongst crop metabolites compositions comprising total acylcarnitine, phospholipids, glucose, organic acids, amino acids, amines and choline of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax), and soil nutrients composition, cation exchange capacity (CEC) and estimated nitrogen-release (ENR) as influenced by variation in frequency (annual, biennial and control) of compost quality assurance tested municipal solid waste compost application. AN, BI, NO are annual, biennial and no (control) compost application, respectively. Projection of the variables in the 2-D factor space (F1 and F2) explained a total of 100% of the variations in the dataset. Variables that are closely located are not different compared to variables located at a distant within a quadrant or between quadrants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

amino acids, amines and choline were similarly influenced by all the soil micronutrients except Al and most of the soil macronutrients, organic matter and ENR (Fig. 5).

Although the pattern of change in total phospholipids content of the edible portions of the vegetable plants was found to be similar to those of the amino acids, acylcarnitine, amines and choline contents, it also strongly and positively association with soil Al. It was previously demonstrated by Lopez-Bucio et al. (2000) that high soil Al content enhanced the accumulation of organic acids in plants for internal Al tolerance. This previous finding agreed with the PCA biplot that confirmed an increase in organic acids contents of the lettuce, green beans and beets in both the annual and the biennial MSW applied plots where Al content was also high. Thus, plant glucose content had a strong negative association with soil Al content, and a moderate positive association with soil CEC and Ca but not the other soil quality indices. Both total plant phospholipids and total organic acids contents showed strong negative association with soil pH, Mg, Ca and CEC. It is worth noting that although the compost applied had high Al content (Table 1), the Al content in the cultivated soils were low (Table 1) leading to a significantly low bioaccumulation factor of between 0.001 and 0.006 for beets, carrot and green beans; and between 0.042 and 0.052 for lettuce (Abbey et al., unpublished). Furthermore, different plant species have different capacity to absorb and utilize soil nutrients and other chemical compounds that determine primary metabolites biosynthesis in plants (Guyonnet et al., 2017). In this study, the chemical composition of the annual, biennial and control (no compost) applied soils, and their specific influence on soil microbial activities in relation to soil-plant interactions may be significantly different (unpublished data). Therefore, the consequential metabolomic manifested differently in the different edible portions of plants for root vegetables (beets and carrot), which was different from the leafy vegetable (lettuce) and pulse (green beans). Plant nutritional strategy is important for the synthesis of primary metabolites required for plant survival, growth, and development. In the present study, annual compost application increased plant uptake of nutrients, particularly N, P and K and some micronutrients compared to biennial application and the least for the control plants. Moreover, each of the test plant is unique and have optimum range and minimum nutrients requirement as previously explained for other plants by Guyonnet et al. (2017). Photosynthesis is the starting point for plant metabolism and as such, resource availability including nutrient sufficiency level is critical. For instance, sufficient N, P and K are essential for chlorophyll formation, enzymes activation, and syntheses of carbohydrates, amino acids and proteins, lipids and transportation and allocation of assimilates and various plant metabolic processes (Guyonnet et al., 2017). In summary, Fig. 6 variations in plant nutrition due to differences in frequency of compost application can explain the varied metabolites compositions of the different vegetables.

4. Conclusion

In conclusion, this study revealed that long-term MSW compost application to agricultural soils induces metabolic changes in lettuce, beets, carrot, and green beans. Overall, the annual application of MSW compost was the best in the enhancement of amino acids, phospholipids, acylcarnitine, amines and choline contents but with a reduced glucose content in lettuce beets, carrot, and green beans. These explain the metabolome dynamics induced by long-term application of MSW compost. These novel findings have shown that food plant biofortification can be achieved using MSW compost, which offers cheaper and environmentally sustainable means of improving nutrients density and functional property of food plants to improve human health and wellbeing. Consequently, these results suggested that compost application to plants may be a cheaper alternative to grow and biofortify food plants to promote healthy diets and human wellbeing. However, further studies involving enzymatic assays and molecular techniques are required to elucidate the mechanisms underpinning such biofortification in response to compost utilization in agriculture.

CRediT authorship contribution statement

Lord Abbey: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Reviewing and editing. **Raphael Ofoe:** Formal analysis, Investigation, Writing - original draft. **Lokanadha Rao Gunupuru:** Validation, Methodology, Writing - reviewing and editing. **Mercy Ijenyo:** Formal analysis, Methodology, Writing - reviewing and

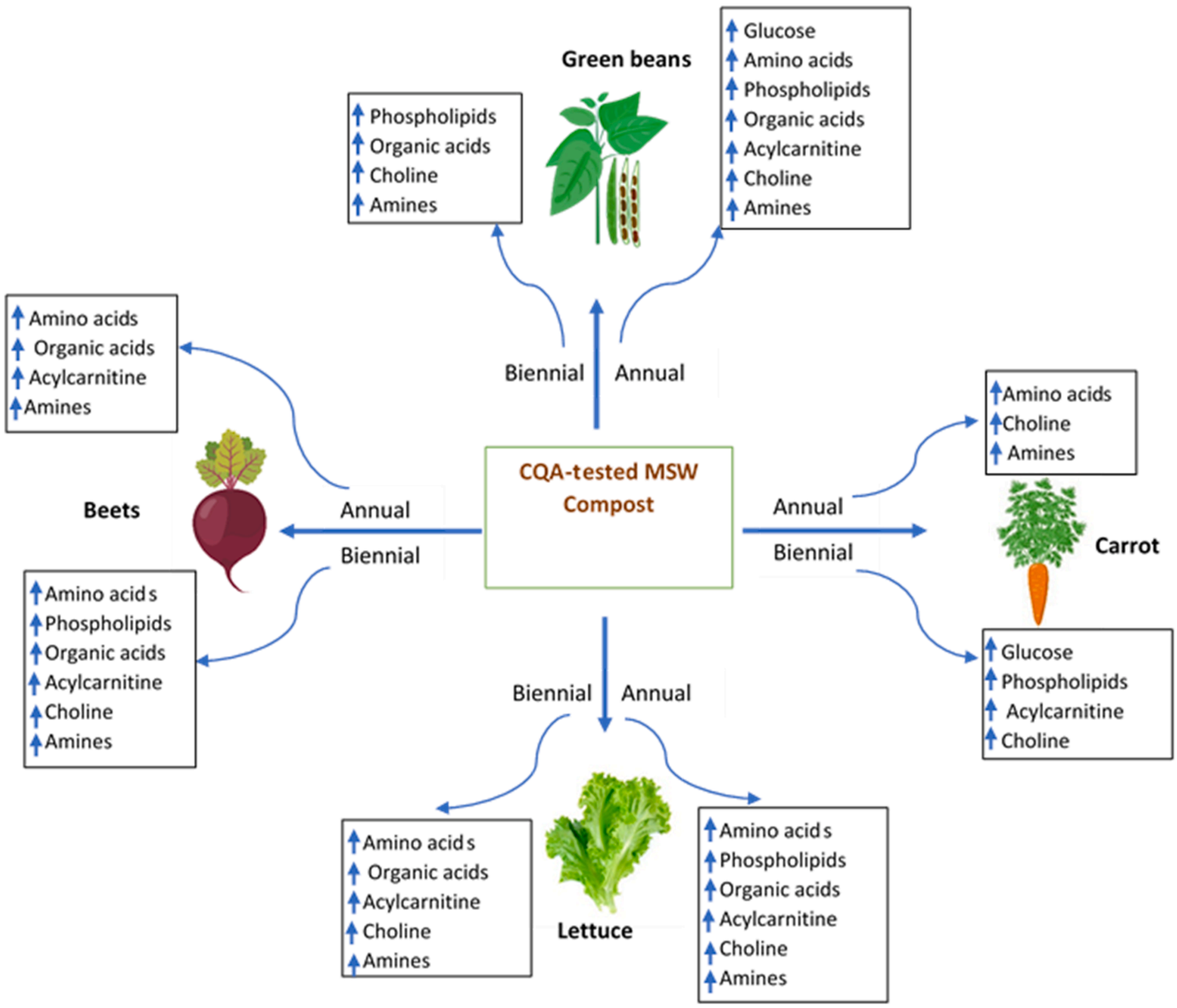

Fig. 6. Summary of metabolic responses of the edible portions of lettuce (*Latuca sativa* cv. Grand Rapids), beets (*Beta vulgaris* cv. Detroit Supreme), carrot (*Daucus carota* cv. Nantes) and green beans (*Phaseolus vulgaris* cv. Golden Wax) as influenced by variation in frequency (annual, biennial and control) of compost quality Alliance (CQA) tested municipal solid waste (MSW) compost application. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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