

Impact of inoculation of the sap collection system with three *Pseudomonadota* strains on maple syrup microbial and organoleptic quality

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Abstract

As it flows through the collection system, maple sap is likely to be contaminated by microorganisms that colonize the tubing, potentially compromising its quality in terms of physicochemical properties, microbial load, and flavor. This study investigates the effect of microbial inoculation, as protective cultures, on the sap collection system to improve maple syrup quality. The research explored how inoculating collection tubing with specific bacterial strains influences the microbial composition, physicochemical properties (pH, Brix, conductivity, sugars, and organic acids content), and sensory attributes of both maple sap and syrup. Three strains selected for their capacity to produce biofilm on plastic tubing and their impact on maple syrup production from inoculated sap, *Pseudomonas* sp. MSB2019, *Janthinobacterium lividum* 100-P12-9, and *Pseudomonas fluorescens* ATCC 17926, were inoculated to independent sap collection system throughout two sugaring seasons. A non-inoculated system was included. *Pseudomonas* sp. MSB2019 treatment resulted in a distinct bacterial composition in sap and impact the organoleptic properties of syrup by the end of second flow season, particularly the maple and overall flavor intensity scores were higher. While sap yield and primary microbial load remained unaffected, inoculation treatments corresponded to shifts in flavor attributes of the syrup. These findings indicate that inoculating sap collection systems with targeted strains can positively influence maple syrup quality, particularly in enhancing desirable flavor profiles, suggesting promising applications for syrup production.

Key words: sap, *Pseudomonas*, *Janthinobacterium*, organoleptic properties, flavor, fungi

Introduction

Maple syrup, a high-value food product, is derived from the concentration of sap collected primarily from the sugar maple tree (*Acer saccharum* Marsh.), and to a lesser extent, from other maple species (Saraiva et al. 2022). The province of Quebec accounts for approximately 90% of Canadian maple syrup production and 67% of the global production (PPAQ 2023a). In Quebec, bulk maple syrup undergoes quality control by an independent agency, with assessments based on syrup color and organoleptic evaluation (taste and smell by inspectors). Maple syrup typically has a Brix of 66–68.9 °Bx and an average water activity of 0.848 (PPAQ 2018). Defects in color and flavor, such as darker color and off-flavors, can result in significant economic losses (N'guyen et al. 2018; PPAQ 2023b).

Maple sap is a nutrient-rich medium that supports the growth of bacteria, molds, and yeasts (Gad et al. 2021; N'guyen et al. 2022). It contains sucrose, other polysaccharides, vitamins, minerals, amino acids, organic acids, phytohormones, and phenolic compounds (Gad et al. 2021). Sap contained microorganisms originating from the tapping

process (Morselli and Whalen 1991). Recent metataxonomic analyses using high-throughput sequencing and culturomics have enhanced the characterization of sap complex microbiota, which is predominantly composed of *Pseudomonadota* (N'guyen et al. 2022; Gupta et al. 2024). The most abundant bacterial genera identified include *Pseudomonas*, *Rahnella*, *Janthinobacterium*, *Hafnia*, *Yersinia*, *Leuconostoc*, *Carnobacterium*, *Cryobacterium*, *Sanguibacter*, and *Gluconobacter*. Although yeasts and molds are frequently present in maple sap, they occur in lower abundance compared to bacteria, with *Mrakia*, *Tausonia*, *Cadophora*, *Leucosporidium*, and *Rhodospiridiobolus* being the most prevalent genera (N'guyen et al. 2022).

Advancements in sap collection methods over recent decades have increased the efficiency and economic feasibility of production. Modern high-vacuum collection systems operate at vacuum levels ranging from 20 to 28 InHg (67.7–94.8 kPa) (Lagacé et al. 2019). Microorganisms originating from the tapping process colonize the tubing collection systems, forming biofilm dominated by *Pseudomonas* spp. (Lagacé et al. 2006a; Perry and Fiore 2022). Consequently, these tubing systems constitute a reservoir of microorganisms, contin-

uously contaminating the sap. The total bacterial load of sap, quantified by qPCR and expressed as gene copy per mL, increases over time rising from approximately 10^6 at beginning of flow period to 10^8 toward the end (Filteau et al. 2012). Specifically, *Pseudomonadota*, *Firmicutes*, and *Actinobacteria* increase due to seasonal temperature fluctuations and compositional changes in the sap associated with tree dormancy release (N'guyen et al. 2022). Microorganisms, particularly yeasts, can elevate the concentration of reducing sugars in sap (glucose and fructose) by the hydrolysis of sucrose, thereby increasing their concentration in syrup, and altering its color and flavor (Lagacé et al. 2002; Nimalaratne et al. 2020; Gad et al. 2021). Strains of *Leuconostoc mesenteroides* can negatively affect the texture of syrup, producing aropy consistency (Lagacé et al. 2018). Moreover, *Leuconostoc* and *Carnobacterium* were associated with buddy off-flavors (N'guyen et al. 2022). On the other hand, certain sap microorganisms are also positively correlated with desirable flavors such as bacteria from the *Pseudomonas fluorescens* group, *Janthinobacterium lividum*, and the yeast *Mrakia*, which are linked to maple and vanilla flavors (Filteau et al. 2011, 2012). Additionally, the maturation of sap at low temperatures with *Pseudomonas geniculata* strains enhanced the characteristic maple flavor of the syrup (Willits et al. 1961).

A natural balance exists within the microbiota of maple sap, with *Pseudomonas* species dominating over lactic acid bacteria, yeasts and molds. To prevent microbial associated defects in maple syrup, there is growing interest in modulating sap microbiota, particularly by controlling biofilm development in tubing collection systems. Different types of biocides have been tested to remove biofilms from tubing (Lagacé et al. 2006b), but currently, isopropyl alcohol (IPA) is the most commonly used (Lagacé 2011). After the sugaring season, IPA is used to sanitize the vacuum sap collection system, reducing microbial load and preparing the system for the next season (Lagacé et al. 2017). However, this process is insufficient to control biofilm formation during the season. An emerging approach for the control of agri-food biofilm involves the use of microorganisms as protective culture to form beneficial biofilms that modulate microbial communities and inhibit the growth of undesirable bacteria (Guéneau et al. 2022). Based on this approach, the aim of this study is to evaluate the impact of the tubing collection system inoculation with three bacterial strains originating from sap on the composition, physicochemical properties, sensory attributes of maple sap and syrup, and the balance of sap microbiota.

Materials and methods

Strain selection and inoculum preparation

A total of 23 strains (19 bacteria and 4 yeasts) were tested for their capacity to produce biofilm on plastic tubing in sap and their impact on maple syrup production from inoculated sap (See Supplementary material, Table S1, S2, and S3, and Fig. S1). Three strains were selected for tubing inoculation in preliminary tests: *Pseudomonas* sp. MSB2019, *J. lividum* 100-P12-9, and *Pseudomonas fluorescens* ATCC 17926. These strains were able to form a biofilm on a piece of tubing with a

high microbial concentration (~ 5 log CFU) without affecting the pH or glucose concentration of the sap (Table S2). Moreover, when the strains were inoculated into sap that was subsequently processed into syrup, the resulting syrups had a physicochemical composition similar to the uninoculated syrup. The strains did not impact glucose concentration, ropiness, viscosity, Brix, transmittance percentage, or syrup acidity (Table S3). *Pseudomonas* sp. MSB2019 (GenBank accession number AY275479) is part of Centre ACER strain collection. *J. lividum* 100-P12-9 (GenBank accession number JN190914) was isolated as in Filteau et al. (2011).

For inoculum preparation, culture glycerol stock stored at -80°C was streaked twice on plate count agar and incubated at 30°C for *Pseudomonas* and 23°C for *J. lividum*. Then, the strains were inoculated in Nutrient Broth No. 3 (Millipore Sigma, St-Louis, MO, USA) to achieve an $\text{OD}_{600\text{nm}}$ of 0.200, and were used at a 1:100 ratio in fresh Nutrient Broth No. 3. This inoculated broth was incubated under mild horizontal agitation (100 rpm) at 21°C for 41–45 h to reach a concentration of 10^6 CFU/mL. The culture broth was centrifuged at $10\,000 \times g$ for 30 min at room temperature. The pellet was washed twice with concentrated sap (8 °Brix) filtrated with $0.2\ \mu\text{m}$ polyethersulfone membrane (Avantor, Radnor, PA, USA). The pellet was then resuspended in 1.7 L of 8 °Brix concentrated sap and partitioned aseptically under a laminar flow hood into 20 mL aliquots under constant agitation, using 30 mL Luer-Lok sterile syringes (BD-Canada, Mississauga, ON, Canada). The syringes were held 1 h at -4°C , then kept frozen at -20°C until tubing inoculation. The inoculum concentrations determined using plate count agar supplemented with 0.5% sucrose incubated for 48 h at 30°C were 4.3×10^8 , 1.87×10^7 , and 3.7×10^8 CFU/mL for *Pseudomonas* sp. MSB2019, *J. lividum* 100-P12-9, and *Pseudomonas fluorescens* ATCC 17926, respectively.

Maple sap collection setup

The experiments were conducted at the sugarbush of Centre ACER, located in Saint-Norbert-d'Arthabaska, Quebec, Canada. New plastic tubing collection systems were installed in two separated experimental blocks within a homogenous area. Trees were randomly selected for each block to ensure a uniform population regarding the diameter at breast height for all collection systems (Table 1). The tree selection was performed at the beginning of the experiment and remained the same afterward throughout the 2 years. The systems in both blocks were identical in terms of supplier, lot, length, arrangement, slope, and exposure, as well as vacuum pump operation, leak correction, and tapping procedures. The collection system installation, including a sanitation of new tubing with IPA prior to their installation, and operation were performed according to general recommendations of North American Maple Syrup Producers Manual (The University of Vermont 2022) and "Cahier de transfert technologique en acériculture" (Houde 2024).

Inoculation and sap collection

Each strain was inoculated into independent sap collection system in the two blocks (Table 1). Approximately 2 weeks

Table 1. Description of the distribution of inoculation treatments in the sap collecting system C and D in the sugar bush.

	Block 1				Block 2			
Sap collection system	C1	C2	C3	C-Control	D1	D2	D3	D-Control
Strain inoculated*	PS	JL	PF	–	PS	JL	PF	–
Number of tapped trees	40	40	40	40	40	40	40	40

*PS: *Pseudomonas* sp. MSB2019; JL: *Janthinobacterium lividum* 100-P12-9; PF: *Pseudomonas fluorescens* ATCC 17926.

prior to the first sap flow, the syringes containing the inoculum were thawed overnight at 4 °C. The 20 mL inoculum was injected into the tapping spout, which was maintained vertically. The spout was then installed in the taphole, and a vacuum level of 27 InHg (91.4 kPa) was applied. This vacuum level was maintained throughout the sap flow season for both years (2016 and 2017), with daily inspections for potential leaks (Lagacé et al. 2019). As it is a standard practice in the maple industry, the collection system was not cleaned within the sugaring seasons. At the end of 2016 season, the collection system was filled with IPA as recommend by St-Pierre (2014) to reduce the biofilm formed. At the beginning of 2017 flow season, inoculation procedures were repeated following the same protocol as the previous year. Daily sap flow was evaluated with calibrated water meters, and each collecting system was connected to a separate extractor for retrieving fresh sap. For both seasons, sap sampling occurred five times during each season (March–April): twice at the beginning (D1 and D2), once mid-season (Mu), and twice at the end (F1 and F2). For each sampling, 150 L of sap was collected in a sterile container and kept frozen at –20 °C until further analysis.

Maple syrup production

Before syrup production, sap samples were concentrated to 12 °Brix using membrane filtration. Two identical membrane concentration systems (Équipements Lapierre Inc. St-Ludger, QC) equipped with four NF-90 type membranes (Filmtec, Minneapolis, MN, USA) were used. To minimize contamination between inoculation treatments, the same membranes were used for the same treatment throughout the season. The membranes were rinsed with hot water, followed by cold water, and a new Purtrex 5 µm pre-filter was used for each concentration. Concentrated sap was stored frozen at –18°C until syrup production. After each concentration cycle, the membranes were rinsed with hot and cold water, followed by an alkaline wash using Ultrasil 10 (Ecolab, Mississauga, ON, Canada), containing tetrasodium EDTA, sodium hydroxide, and organic sulfonic acid salt. A new pre-filter was installed, and the system was rinsed with hot and cold water again.

The sap samples were thawed uniformly over a 24 h period in a recirculating water tank at temperatures ranging from 4 to 8 °C depending on the thawing stage, allowing a slow and uniform process and to prevent microbial growth as described by Lagacé et al. (2019). Then sap sample were being processed by a pilot-scale evaporator with three folded pans and three flat-bottomed pans, providing a total evaporation surface of 270 sq. ft (25 m²). The sap was evaporated until 66

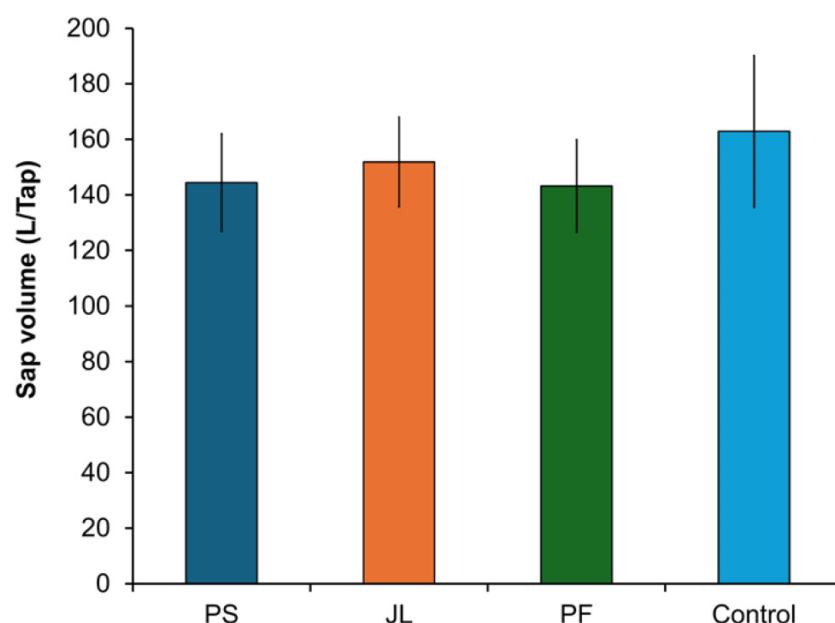
°Brix. Freshly made syrup was transferred to a double-wall syrup maker with a closed lid to retain heat. The syrup was filtered through a pilot-scale filter press equipped with vertical plates, applying pressure ranging from 15 to 30 psi (103.4–206.8 kPa). The syrup was then immediately poured hot into sterile glass containers. After cooling, the syrup samples were stored frozen until further analysis.

Physicochemical, microbial, and sensorial analyses

Maple sap and syrup samples were thawed in the laboratory for analysis. Measures of pH, Brix, conductivity, sugars and organic acids content were performed following the methods described by Lagacé et al. (2015). Total aerobic mesophilic bacteria counts (TBC) and total fungal counts (TFC) of sap samples were determined using viable counts (Lagacé et al. 2019). Sap samples diluted into peptone water (0.1%, w/v) and appropriate dilutions were plated on plate count agar (BD-Canada) which were incubated for 48 h at 30 °C for TBC. TFC were obtained by plating on Dichloran Rose Bengal Chloramphenicol Agar (BD-Canada) incubated for 5 days at room temperature. All microbial counts were expressed in colony forming units per mL of raw maple sap (CFU/mL) with log values used for calculations. Syrup color intensity was assessed by measuring light transmittance at 560 nm (%T) in a Genesys 20 visible spectrophotometer (ThermoFisher Scientific, Canada), with glycerol as a reference. Color grading followed the most recent provincial standards (Gouvernement du Québec 2024).

The quality of the syrup samples was evaluated by certified inspectors following standard procedures established in the Quebec maple industry (PPAQ 2023b). These inspectors are specifically trained to assess maple syrup quality and are skilled in detecting flavor defects. This analysis was conducted to determine the possible impact of the treatments on the product's commercial value. Then, a sensorial analysis was performed by Agriculture and Agri-food Canada with a panel of 20 untrained participants, using the deviation from reference profile (ISO 13299:2016). Labeled control syrup and four test samples heated at 35 °C were presented to the participants, with a possible blind control included. The panel members evaluated the magnitude of difference between control and test samples, as well as maple flavor attributes. The global flavor (overall positive assessment) and off-flavors were evaluated with a nine-level categorical scale (very light to high) with the possibility to be equal as the control. Off-flavors were described by specific terms: cardboard, spicy, astringent, metal, and packaging. Each test followed a

Fig. 1. Sap volume per tap (L/tap) of tubing collection system inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 compared to control (non-inoculated), across two flow seasons. Error bars represent standard deviations from the mean.



Latin square design. Data acquisition and statistical analysis using Dunnett's test were performed with Fizz software version 2.4 A (Biosystem, Couternon, France).

Metataxonomic analysis of sap

The microbiota of sap collected from Block 2 were characterized at three sampling periods (D1, Mu, and F2). A volume of 1 L of sap was centrifuged at $12\,000 \times g$ for 20 min at 4°C , and the pellet was transferred in a 50 mL conical tube for further centrifugation at $6076 \times g$ for 15 min at 4°C . DNA was extracted from the pellet using the DNeasy PowerWater Kit (Quiagen, Hilden, Germany). Sequencing was performed in a single run on Illumina MiSeq at the Plateforme d'Analyses Génomiques, IBIS Université Laval (Québec, Canada). Generic primer pairs were used to amplify the V3-V4 region of the 16S rRNA gene for 2×300 pb paired-end sequencing (Klindworth et al. 2013).

Bioinformatics analysis was conducted using Microbiome-Analyst version 2.0 (Lu et al. 2023). Amplicon sequencing data were processed via the Raw Data Processing module, employing the DADA2 workflow and Greengenes taxonomy. This analysis included filtering, dereplication, sample inference, chimera identification, and the merging of paired-end reads. Quality control parameters were rigorously applied, with a forward truncation length of 289 bp and a reverse truncation length of 249 bp, a maximum expected error of 2 for both forward and reverse reads, trimming of 10 bases from each end, a maximum allowable N count of 0, minimum quality score of 1, truncation quality of 2, and PhiX sequence removal. The resulting amplicon sequence variant (ASV) abundance table was analyzed using the Marker Data Profiling module for further data analysis and visualization. Data normalization was achieved through a low-count filter (≥ 4 counts with 10%

prevalence), a low-variance filter (10% based on interquartile range), and cumulative sum scaling. Dendrogram analysis utilized the Bray-Curtis distance measure and Ward's clustering algorithm. For beta diversity profiling, the Principal coordinate analysis (PCoA) ordination method and Bray-Curtis distance were employed, with pairwise PERMANOVA analysis to compare group differences. Multitesting adjustment was performed using the Benjamini-Hochberg procedure (FDR). Identification of significant features, potentially indicative of biomarkers, was carried out with the random forest machine learning algorithm, with the number of trees set at 500 and the number of predictors set at 7.

Statistical analysis

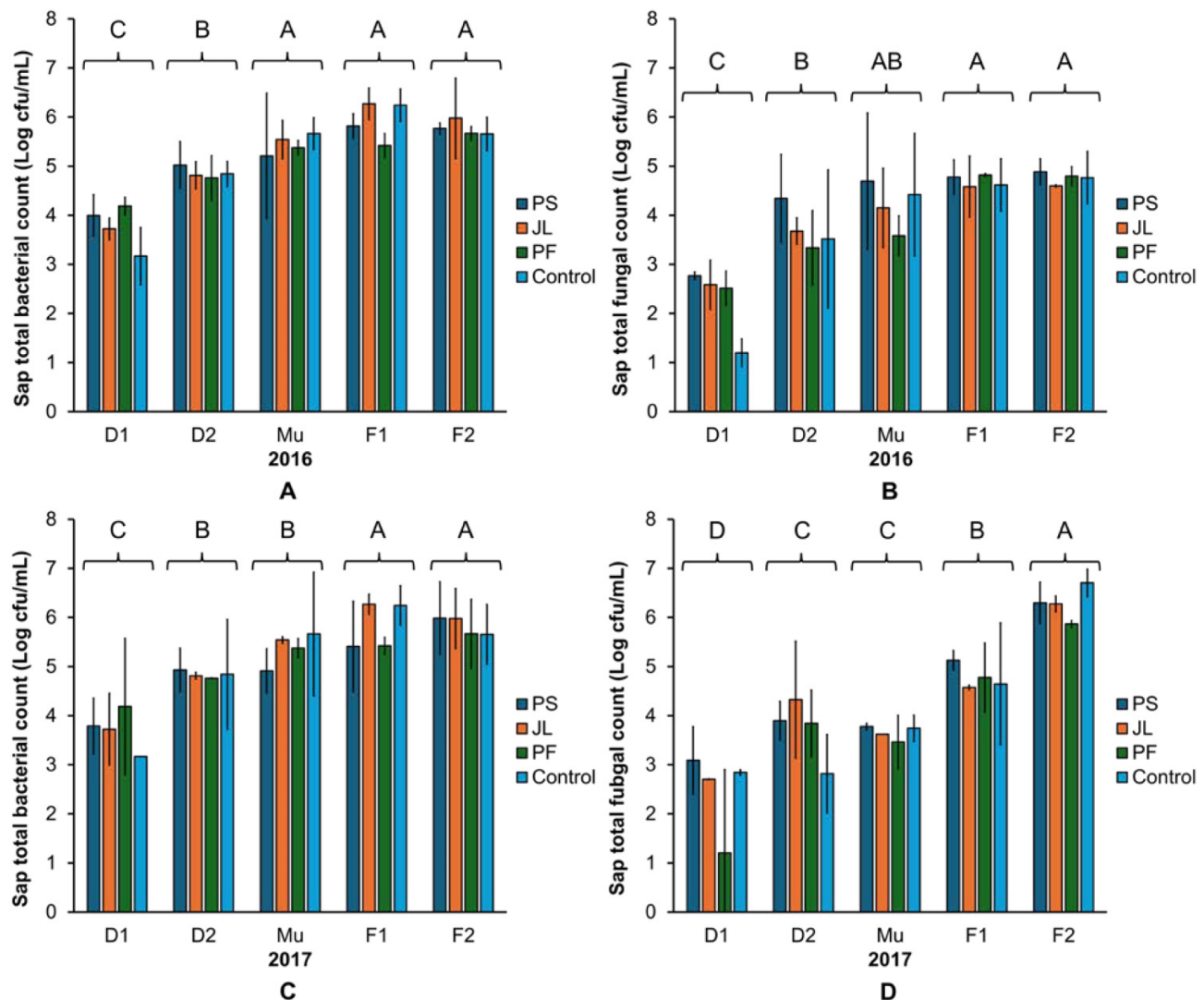
To assess significant differences between treatments, XL-STAT software equipped the multiple linear regression model was used. Normality and homogeneity of variance were evaluated with Shapiro-Wilk's test and Levene's test, respectively. ANOVA was performed and means were compared with Duncan's multiple comparison test.

Results

Effect of inoculation on sap yield

The sap flow yield across the three inoculated systems and the non-inoculated control showed no statistically significant difference ($P = 0.604$; Fig. 1). The sap volume ranged from 143.21 L per tap in the system inoculated with *Pseudomonas fluorescens* ATCC 17926 to 162.86 L in the non-inoculated system. This indicates that the inoculation of the sap collection systems with the selected bacterial strains had no measurable

Fig. 2. Total bacterial count (A, C) and total fungal count (B, D) in sap at the beginning of the flow (D1, D2), mid-season (Mu), and end of the flow (F1, F2) during 2 years of experimentation. The tubing collection system was inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 (PF), compared to the non-inoculated control. Error bars represent standard deviations from the mean. Different letters indicate significant differences ($P < 0.05$).



impact on sap yield during the experiment. Blockage could have occurred due to massive biofilm formation.

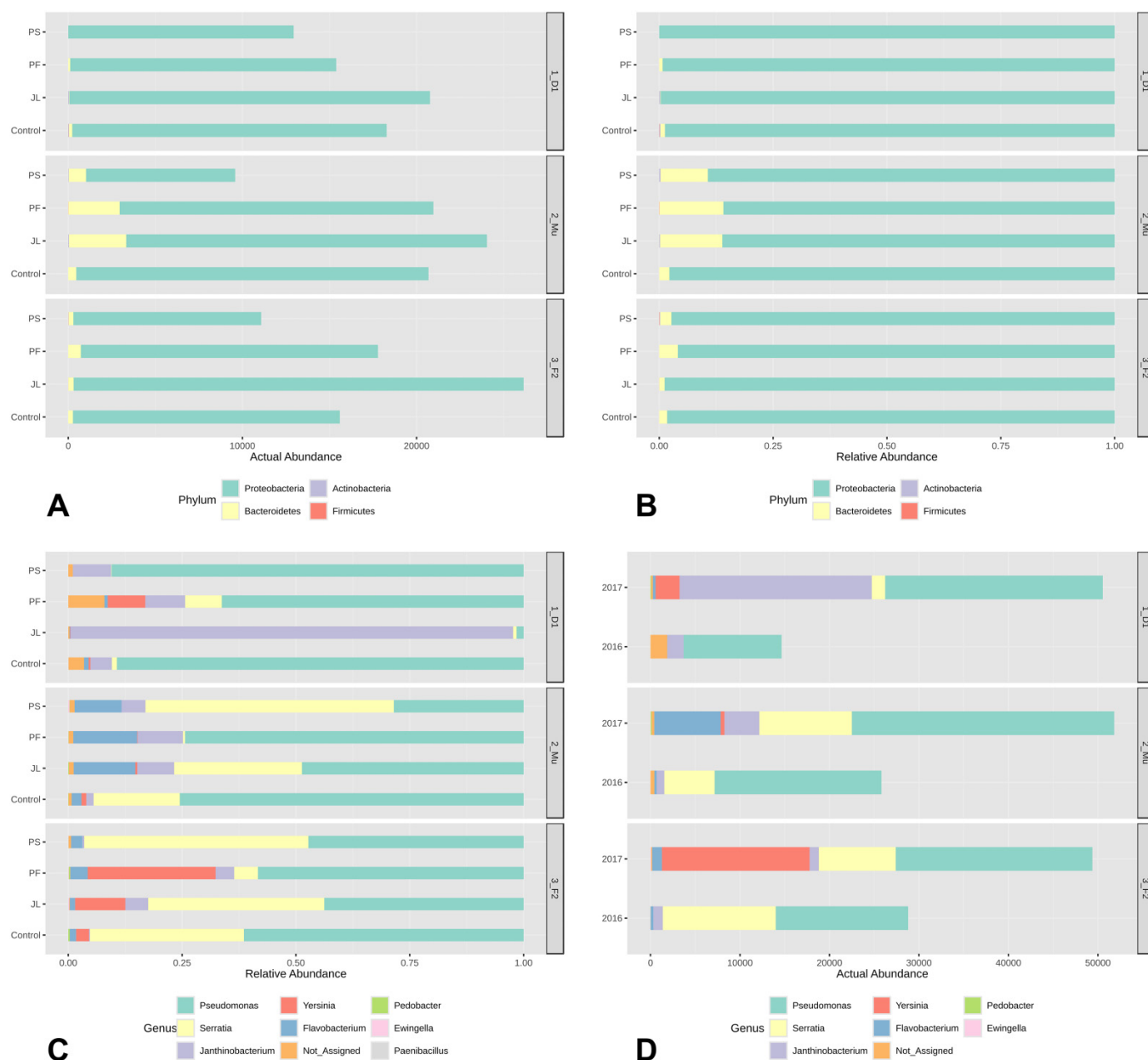
Microbial characterization of sap

For both years, the TBC in sap increased progressively throughout the flow season (Figs. 2A and 2C). A similar pattern was observed in both 2016 and 2017, with a significant rise in TBC from the initial sampling (D1) to mid-season (Mu) ($P < 0.0001$), after which a plateau was reached at the final sampling (F2) ($P = 0.506$). The standardized coefficients of the linear model of the statistical analysis are presented in Table S4. Microbial populations increased in sap samples as the season progressed. At the beginning of the flow, the bacterial load in sap was 3.55 log CFU/mL, increasing by approximately 2 log to reach 5.96 log CFU/mL by the season's end. Multiple comparisons of means did not show a significant impact of

the collection system inoculation on the TBC. The TFC in sap also increased over time, though the pattern differed slightly between the 2 years of experimentation (Figs. 2B, 2D, and Table S5). In 2016, the fungal population reached its final concentration earlier than in 2017. Additionally, the fungal load at end of the flow season was lower in 2016 compared to 2017, corresponding to 4.76 and 6.28 log of CFU/mL, respectively. In 2016, at the first sampling period, the TFC of sap in the control system was lower than in the inoculated systems (1.2 vs. 2.62 log CFU/mL). However, no significant global difference was observed between the inoculated and non-inoculated systems ($P > 0.143$).

From the 24 samples (2 years, 4 conditions, and 3 sampling periods), high-throughput sequencing produced a total read count of 236 001, with an average of 10 260 reads per sample, a maximum of 20 779, and a minimum of 249.

Fig. 3. Bacterial abundance in sap collected at the beginning of the flow (D1), mid-season (Mu), and end of the flow (F2) across 2 years of experimentation. The tubing collection system was inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 (PF), compared to the non-inoculated control (Control). (A) Actual abundance at phylum level, (B) relative abundance at phylum level, (C) relative abundance at genus level, and (D) actual abundance at genus level.



The JL sample at D1 in 2016 was excluded from analysis due to unsuccessful paired-read merging. After data normalization, six phyla, six classes, seven orders, seven families, eight genera, and six species have been identified after data normalization (Fig. 3 and Supplementary Fig. S2). The phylum *Pseudomonadota* was dominant in all sap samples, comprising 95.97% of reads, followed by *Bacteroidetes* (4.01%), *Actinobacteria* (0.02%), and *Firmicutes* (0.01%) (Figs. 3A and 3B). Sap samples from tubing inoculated with *J. lividum* exhibited the highest read counts across sampling periods, whereas samples inoculated with *Pseudomonas* sp. MSB2019 had the lowest.

By mid-season, *Bacteroidetes* proportions increased reaching to 10%–14% in inoculated treatments but declined by season's end, while control treatments maintained stable levels (1%–2%) throughout sampling. At the genus level, sap from tubing inoculated with *Pseudomonas* sp. MSB2019 at the season's onset (D1) had a microbiota profile similar to the control (Fig. 3C). *Janthinobacterium* initially dominated the D1 sap samples inoculated with JL, but its relative abundance returned to levels comparable to other inoculated treatments by mid-season (8%). At the season's end (F2), *Janthinobacterium* levels in PS and control treatments dropped to 0.4% and 0.2%, respectively.

Notably, only sap samples associated with the PS treatment lacked *Yersinia* across all sampling periods, while its abundance increased over time in other treatments. In PF-treated sap at the season's end, *Yersinia* reached 28% relative abundance. *Serratia* was prevalent in mid- and late-season sap samples, except in those associated with *Pseudomonas fluorescens* ATCC 17926.

The sap collected from PS-treated tubing systems showed clustering within the same year of collection (Fig. S3). Alpha diversity indices, including Chao1, Shannon, and Simpson, did not significantly differ between the inoculated and non-inoculated tubing systems (Fig. 4A). However, for beta diversity profiling, the PCoA revealed a significant effect of inoculation treatment on sap microbiota composition ($P < 0.001$) (Fig. 4C). Pairwise PERMANOVA analysis demonstrated that the microbiota associated with PS treatment differed significantly from that of other treatments, including the control ($P < 0.01$). In contrast, inoculation with JL and PF did not lead to significant changes in sap bacterial composition.

The random forest algorithm identified distinctive microbial features across treatments (Fig. 5). At the genus level, *Janthinobacterium* was strongly associated with the JL treatment, with moderate associations observed for *Yersinia*. In the PF treatment, *Flavobacterium*, *Yersinia*, and *Paenibacillus* were highly associated, while *Janthinobacterium* and *Pseudomonas* were moderately associated. *Pseudomonas* and *Serratia* emerged as significant features of the PS treatment. At the ASV level, no bacterial biomarkers were shared between treatments. Sap from the non-inoculated system was strongly associated with two ASVs of *Pseudomonas* sp. and one ASV of *Pseudomonas veronii*. Inoculation with JL correlated strongly with three ASVs of *Janthinobacterium lividum* and one ASV of *Pseudomonas umsongensis*. PF-treated sap samples showed high associations with six distinct ASVs, including two *Pseudomonas veronii* ASVs, one *Pseudomonas umsongensis* ASV, and three ASVs of *Pseudomonas* sp. Lastly, two ASVs of *Pseudomonas* sp. were strongly associated with PS inoculation.

The microbiota of sap differed significantly between 2016 and 2017 (Fig. 3D), with read counts in 2017 being twice as high as those in 2016. Genera such as *Ewingella*, *Pedobacter*, and *Yersinia* were only detected in 2017. While the Chao1 alpha diversity index revealed a significant difference between 2016 and 2017 ($P < 0.01$), no significant differences were observed in the Shannon or Simpson indices (Fig. 4B). Beta diversity analysis indicated a significant distinction between sap samples from 2016 and those from 2017 ($P < 0.05$) (Fig. 4D). Interestingly, the dendrogram of sap microbiota did not group samples by year, nor did it cluster samples based on collection timing within the flow season (Fig. S3). Additionally, beta diversity did not significantly vary according to the sampling period factor (Fig. S4).

Physicochemical characterization of sap and syrup

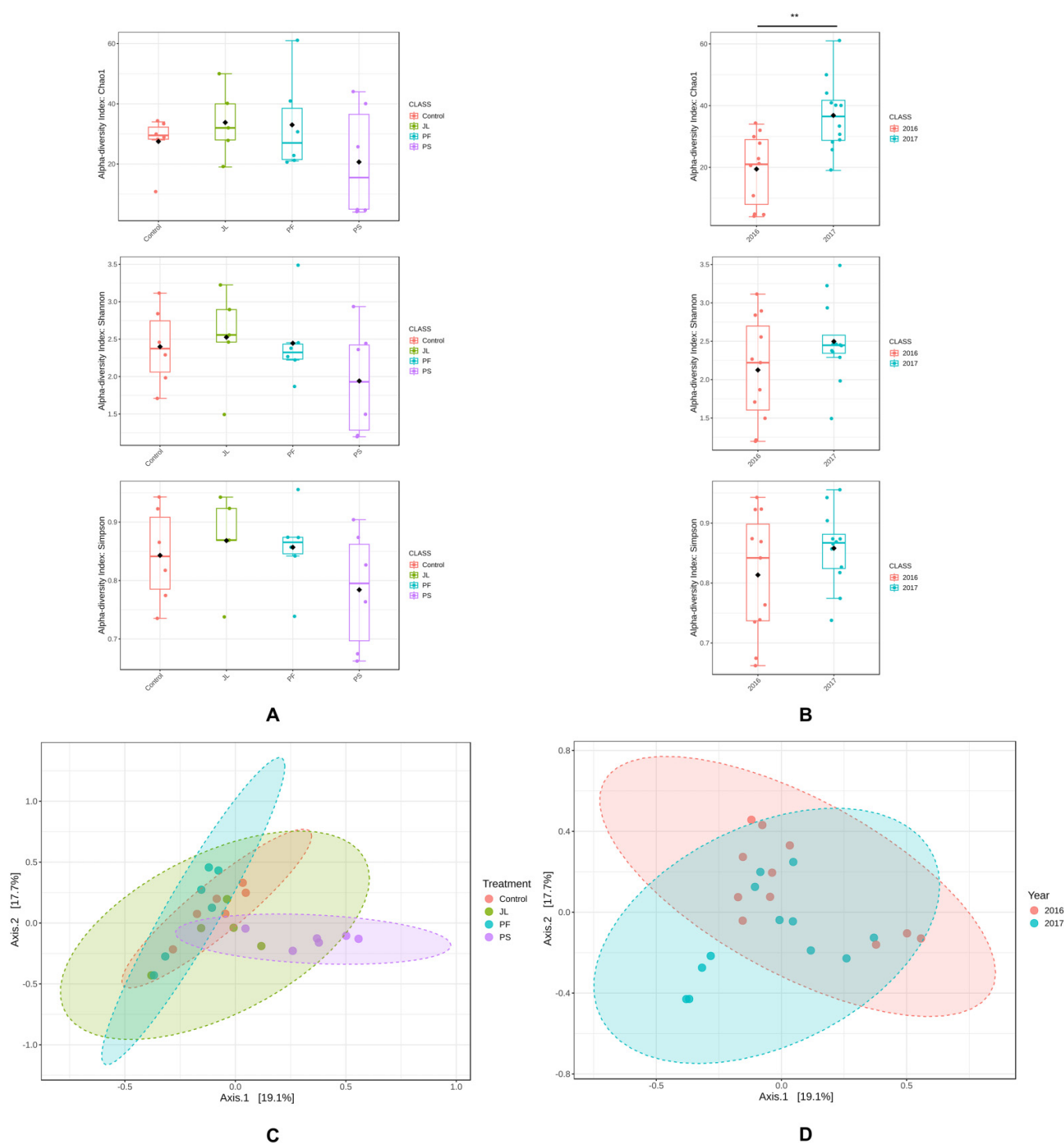
Several physicochemical parameters of sap and syrup samples were evaluated to assess potential degradation. The pH of the sap was not significantly affected by the year of harvest or inoculation treatment ($P > 0.05$), but it varied throughout

the sampling periods (Fig. 6A and Table S6). At the beginning of the flow, the sap pH was 7.65, declining in mid-season at 7.21, and decreasing further to 6.55 by the end of season. Despite this decline in sap pH over time, the pH of the syrup was unaffected by the different treatments ($P = 0.30$) (Fig. 6B). In contrast to pH, sap conductivity was significantly influenced by the harvest year ($P < 0.0001$). Conductivity was lower in 2016 (501.1 $\mu\text{S}/\text{cm}$) than in 2017 (554.9 $\mu\text{S}/\text{cm}$). A similar difference was observed in syrup, with conductivity values of 185.0 and 218.3 $\mu\text{S}/\text{cm}$ for 2016 and 2017, respectively. Conductivity of sap increased significantly throughout the season, rising from 449.2 $\mu\text{S}/\text{cm}$ during the first sampling period to 528.5 $\mu\text{S}/\text{cm}$ in the second period (Fig. 6C and Table S7). By the end of the season, conductivity was significantly higher compared to the beginning (D1). In syrup (Fig. 6D), conductivity was also significantly higher at the end of the season (220.3 $\mu\text{S}/\text{cm}$) compared to the beginning and mid-season (189.2 $\mu\text{S}/\text{cm}$). There was no impact of inoculation on the conductivity of sap and syrup samples. The light transmittance of the syrup samples was measured (Fig. S5). There was no significant impact of either the year or the inoculation treatment ($P > 0.05$). However, the percentage of transmittance at 560 nm decreased from 82.41% to 76.54% after mid-season. Even at the lower light transmittance value, the maple syrup samples were color-classified in the greatest category (e.g., Golden–delicate taste).

The major carbohydrate in sap, sucrose, was not significantly different between the 2 years of experimentation ($P = 0.158$), but its concentration varied throughout the flow season (Fig. 6E and Table S8). The sucrose content was at 2.24% (w/w) at beginning of the season, increasing to 2.59% at second sampling, which represented the peak concentration. The sucrose content then decreased progressively, reaching 1.84% at the end of season. The Brix value in sap sample was similar to the sucrose content (Table S9). Interestingly, sap collected from the control system contained more sucrose than that from the inoculated system (mean: 2.36% vs. 2.19% w/w). However, there was no significant difference in the sucrose content of syrup produced from these sap samples, with a mean concentration of 64.36% (Fig. 6F). Glucose and fructose contents in sap were relatively low, with several samples below the detection limit (Table 2). Glucose content was significantly higher in 2017 compared to 2016, at 0.0033% (0.1831 mmol/L) and 0.0014% (0.0777 mmol/L), respectively ($P = 0.006$). In 2016, fructose content in sample was mostly below the detection limit, while in 2017, it reached 0.003% (0.1665 mmol/L). Both reducing sugars were significantly higher at the end of season (F2), with no observed effect of inoculation. There was no significant difference in the concentrations of glucose and fructose in maple syrup samples across all inoculation conditions ($P = 0.212$ and $P = 0.081$, respectively). As in sap samples, concentrations of reducing sugars were significantly higher at end of the flow compared to other sampling periods. The Brix value of syrup samples was higher than sucrose content as it also includes glucose, fructose, organic acids, and minerals (Table S9).

A total of 11 organic acids were measured in sap and syrup samples (Table S10). Malic acid was the most concentrated organic acid in both the sap and the syrup, with concentrations

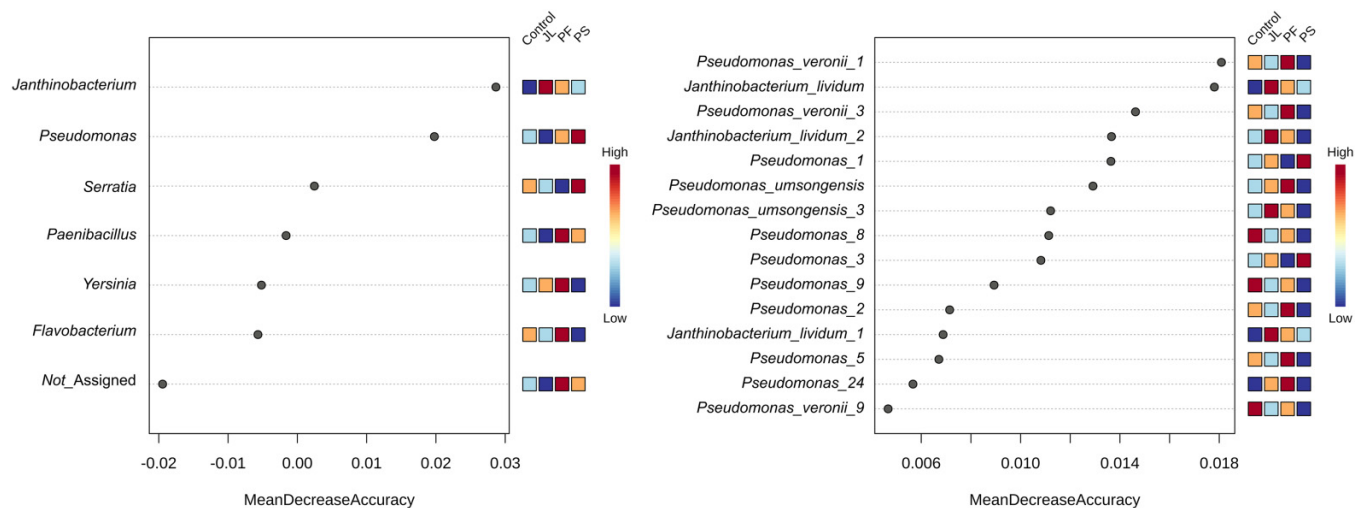
Fig. 4. Comparison of alpha and beta diversity measure of sap. The alpha-diversity index box plots (Chao1, Shannon, and Simpson) regarding inoculation treatment (A) and year of experiment (B). Asterisks indicate significant differences in the alpha diversity indices based on the Welch *t* test/ANOVA (***P* value < 0.01). Principal coordinate analysis (PCoA) of Bray–Curtis similarities calculated based on relative abundances at feature-level regarding inoculation treatment (C) and year of experiment (D). The tubing collection system was inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 (PF), compared to the non-inoculated control (Control).



of 355.47 $\mu\text{g/g}$ in the sap and 4812.82 $\mu\text{g/g}$ in the syrup. There was no significant difference between treatments and the 2 years of experimentation (Table S10). Acetic, quinic, and succinic acid concentration in sap were significantly higher in

2017 compared to 2016 (Table 3). Citric acid showed the opposite trend. In syrup, acetic acid was seven times higher in 2017, while citric acid was more concentrated in 2016, similar to the sap. Surprisingly, the quinic acid in syrup did not have

Fig. 5. Random forest analysis on sap microbiota collected from collection systems non-inoculated (Control) and inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 (PF). The most important taxa for the treatment classification were ranked by their importance from top to bottom.



the same tendency as in the sap and was lower in 2017. Pyruvic acid concentration in syrup was 1.5 times lower in 2017. As with acetic acid, lactic and oxalic acid levels were higher in 2017. Shikimic acid was detected only in syrup samples from 2017, while tartaric acid was detected only in 2016. Inoculation had no significant impact on the concentrations of these 11 organic acids in either sap or syrup samples.

Sensory characteristics of the syrup

Syrup samples from both the inoculated and control systems were first evaluated by an inspection service to assess the presence or absence of defects (Table 4). All syrup samples produced in 2016 were free of flavor defects, exhibiting compliant taste profiles with only occasional traces of defect that did not warrant penalties. In 2017, however, the syrup samples obtained from sap from the control system exhibited off-flavors at both the beginning (D1) and end (F2) of the season, being classified as VR4 and VR1, respectively. The VR4 defect indicate an unidentified flavor defect, while VR1 is associated with natural flavor defects, such as those related to wood, sap, burnt, or over-caramelized notes. Syrup samples produced from inoculated sap collection systems in 2017 were all presenting absence or slight trace of flavor defect (✓).

The sensory evaluation performed by a panel of 20 participants for the 2016 syrup samples revealed a significant different in the intensity of maple flavor at the end of season (F2) (Table 5). The intensity of the maple flavor was lower in syrup samples associated with *Pseudomonas* sp. MSB2019 inoculation compared to the control. In contrast, in 2017, the inoculation had a more pronounced impact on syrup flavor. At the first sampling period (D1), an off-flavor was detected in syrup associated with *J. lividum* 100-P12-9 and *Pseudomonas fluorescens* ATCC 17926 inoculations, which exhibited a higher intensity of cardboard packaging-like taste. At F1 sampling period, syrup samples treated with *Pseudomonas* sp. MSB2019 had a more intense global flavor compared to those

associated with *J. lividum* 100-P12-9 and *Pseudomonas fluorescens* ATCC 17926 treatments. At last sampling period (F2), the inoculation with strains MSB2019 and 100-P12-9 demonstrated higher intensities of both global flavor and maple flavor compared to the control.

Discussion

The inoculation of the sap collection tubing system did not appear to increase the rate of tubing colonization by microorganisms compared to the control, as the microbial load in the sap was similar regardless of inoculation status. Excessive microbial development could have restricted sap circulation (Lagacé et al. 2006b). The mean sap yield per tap (150.59 L) in the present study was comparable to that reported by Lagacé et al. (2019), which was 163.13 L per tap for a collection system operating at a vacuum level of 28 InHg. As noted, the sap's bacterial cultivable load was unaffected by inoculation status. Progressive sap contamination during the flow season was also observed in previous studies (Lagacé et al. 2002, 2015; N'guyen et al. 2022). In future studies, it will be important to validate and quantify biofilm formation in the tubing by the inoculated strains.

The bacterial composition of all sap samples was composed in large proportion of *Pseudomonadota*, specifically *Gammaproteobacteria* and *Betaproteobacteria*, indicating good sap quality, as mentioned in previous studies (Lagacé et al. 2004; Filteau et al. 2012). Alpha diversity indices showed that inoculating the collection system did not significantly alter the bacterial diversity within the sap. As previously described by N'guyen et al. (2022), a normal succession of microorganisms occurs in sap following the release from tree dormancy. In the present study, at mid-season, the proportion of *Flavobacterium succinicans*, a member of *Bacteroidetes*, was higher in sap associated with inoculation to the non-inoculated control. This increase could have resulted from the species' ability to form

Fig. 6. Physicochemical characteristics of maple sap (A, C, E) and syrup (B, D, F) at the beginning of the flow (D1, D2), mid-season (Mu), and end of the flow (F1, F2) across 2 years of experimentation. The tubing collection system was inoculated with *Pseudomonas* sp. MSB2019 (PS), *Janthinobacterium lividum* 100-P12-9 (JL), and *Pseudomonas fluorescens* ATCC 17926 (PF), compared to the non-inoculated control. Error bars represent standard deviations from the mean. Different letters indicate significant differences ($P < 0.05$).

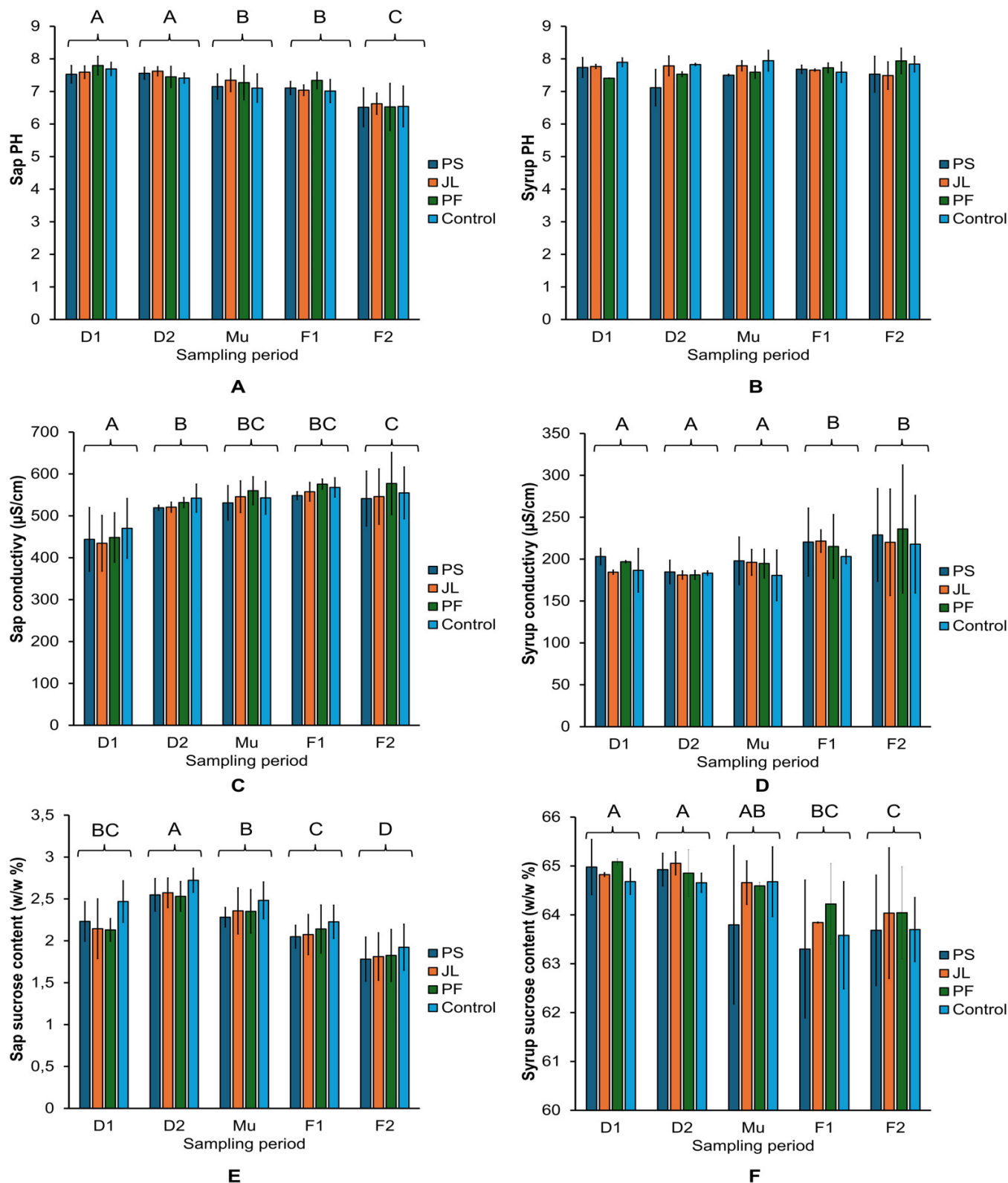


Table 2. Concentration of reducing sugars in maple sap and syrup for flow season 2016 and 2017 (mmol/L $\pm$ standard deviation).

	Period	Glucose		Fructose	
		2016	2017	2016	2017
Sap	D1	<DL	<DL	<DL	0.0110 $\pm$ 0.0110 ^B
	D2	<DL	<DL	<DL	0.0444 $\pm$ 0.0167 ^B
	Mu	<DL	0.0555 $\pm$ 0.0944 ^B	<DL	0.0888 $\pm$ 0.0722 ^B
	F1	0.0777 $\pm$ 0.228	0.0832 $\pm$ 0.0777 ^B	0.0389 $\pm$ 0.1055	0.1221 $\pm$ 0.0999 ^B
	F2	<DL	0.4107 $\pm$ 0.0483 ^A	<DL	0.4218 $\pm$ 0.4363 ^A
Syrup	D1	0.6938 $\pm$ 0.8104	1.5153 $\pm$ 0.1887 ^B	<DL	1.1767 $\pm$ 0.2553 ^B
	D2	1.8150 $\pm$ 0.1776	1.3932 $\pm$ 0.1388 ^B	0.2440 $\pm$ 0.4890	1.1823 $\pm$ 0.2109 ^B
	Mu	0.6161 $\pm$ 0.7216	1.6208 $\pm$ 0.3386 ^B	<DL	1.4210 $\pm$ 0.1943 ^B
	F1	3.5080 $\pm$ 4.7902	1.8928 $\pm$ 0.7138 ^B	1.7164 $\pm$ 3.4327	2.1647 $\pm$ 0.4996 ^B
	F2	1.0879 $\pm$ 0.7937	6.9771 $\pm$ 4.2684 ^A	<DL	4.7957 $\pm$ 2.5977 ^A

Note: DL: Detection limit. Different letters within the same column and separately between sap and syrup samples indicate significant differences ($P < 0.05$).

Table 3. Concentration of organic acids ($\mu\text{g/g} \pm$ standard deviation) in maple sap and syrup which has varied between 2016 and 2017.

	Organic acid	2016	2017	P value*
Sap	Acetic acid	4.27 $\pm$ 5.94	35.10 $\pm$ 19.88	<0.0001
	Citric acid	21.80 $\pm$ 5.36	10.45 $\pm$ 4.18	<0.0001
	Quinic acid	18.543 $\pm$ 42.30	51.75 $\pm$ 82.46	0.038
	Succinic acid	14.7 $\pm$ 11.38	37.52 $\pm$ 48.66	0.009
Syrup	Acetic acid	147.078 $\pm$ 199.19	1021.76 $\pm$ 460.33	<0.0001
	Citric acid	263.37 $\pm$ 55.67	228.59 $\pm$ 44.27	0.01
	Lactic acid	27.073 $\pm$ 24.52	64.27 $\pm$ 52.29	0.002
	Oxalic acid	14.08 $\pm$ 4.36	34.54 $\pm$ 14.32	<0.0001
	Pyruvic acid	229.08 $\pm$ 142.35	158.036 $\pm$ 124.52	0.005
	Quinic acid	48.636 $\pm$ 30.89	18.101 $\pm$ 29.35	0.004
	Shikimic acid	<DL	0.32 $\pm$ 5.60	0.017
	Tartaric acid	48.85 $\pm$ 25.43	<DL	<0.0001

*Statistical comparison between the 2 years. DL, detection limit.

biofilms on pre-existing layers produced in inoculated treatments. Zhang et al. (2013) demonstrated that *Flavobacterium* sp. produced biofilm on the surface of *Pseudomonas aeruginosa* biofilm, potentially enhancing nutrient access. More studies will be needed to confirm this observation.

Overall, microbial quantification along with alpha and beta diversity indices indicate that inoculation of the sap collection system did not disrupt the sap's microbial composition. This stability may result from the selected strains' origins within the maple environment, where they are well adapted to this ecological niche. The present study could not confirm the persistence of inoculated strains. However, the fact that the sap samples from collection system inoculated with *J. lividium* 100-P12-9 were enriched with this species at the beginning of flow season, suggests its capacity to adhere to the tubing system. However, this species was not sufficiently competitive to remain dominant throughout the season. This is not a necessarily negative outcome, as maintaining balance in sap microbiota plays an important role in determining the flavor of maple syrup (Perry and Fiore 2022). For instance, *Pseudomonas* species contribute to the characteristic maple and

vanilla flavors in syrup (Filteau et al. 2012). Thus, the inoculation with *J. lividium* species did not inhibit *Pseudomonas* colonization.

The identification of biomarker species such as *Paenibacillus*, *Yersinia*, and *Flavobacterium* in sap associated with *Pseudomonas fluorescens* ATCC 17926, but absent from control, could suggest that inoculation with this strain favored the presence of these microorganisms in the sap. This effect may be linked to cooperative interactions within biofilms (Li et al. 2021). In contrast, the other *Pseudomonas* strain used in our study had an opposing effect. Indeed, PS-treatment resulted in a distinct bacterial profile in the sap, suggesting that the inoculated strain, *Pseudomonas* sp. MSB2019, colonized the tubing system and competed effectively with other bacteria, including *Yersinia* spp. The presence of *Yersinia* in maple sap was observed by N'guyen et al. (2022) and Gupta et al. (2024). In the study by N'guyen et al. (2022), six OTUs associated with this genus were detected in 11 out of 47 sap samples. The conformity status of the maple syrup associated with these samples included four classified as standard, five as non-standard, and two for which conformity

Table 4. Organoleptic quality assessment of maple syrup samples as evaluated by the inspection service.

Inoculation*	Sampling period	2016		2017	
		Classification [†]	Description	Classification	Description
PS	D1	Ok	Bland	Ok	–
	D2	Ok	–	Ok	–
	Mu	Ok	–	Ok	–
	F1	Ok	–	✓	Slightly acidic
	F2	✓	Slightly wood	✓	Caramelized, slightly acidic
JL	D1	✓	Slight taste at the end	✓	Less sweet, slightly harsh
	D2	Ok	–	✓	Less sweet, slightly acidic
	Mu	Ok	–	Ok	–
	F1	✓	Acid taste	Ok	–
	F2	Ok	–	✓	Caramelized, slightly harsh, slightly acidic
PF	D1	✓	Slightly wood	✓	–
	D2	✓	Paper	✓	–
	Mu	Ok	–	Ok	–
	F1	Ok	Good characteristic flavor	✓	Less sweet, clear in mouth
	F2	Ok	Bland	✓	Bland, harsh
Control	D1	✓	Slightly wood	VR4	Bad smell, harsh, aftertaste
	D2	Ok	–	✓	Bland, slightly harsh
	Mu	Ok	–	Ok	–
	F1	Ok	Good characteristic flavor	Ok	–
	F2	Ok	–	VR1	Caramelized, slightly harsh

*PS: *Pseudomonas* sp. MSB2019; JL: *Janthinobacterium lividum* 100-P12-9; PF: *Pseudomonas fluorescens* ATCC 17926; Control: non-inoculated.

[†]Ok: typical taste of maple, free from unusual taste or odor; ✓: slight trace of undesirable taste and smell; VR: unpleasant taste and smell (PPAQ 2023b).

data were not available. Interestingly, *Yersinia* was detected in all sap samples from one particular producer. It would be valuable to investigate whether specific production practices may favor the presence of this genus. To date, no studies have established a link between *Yersinia* and off-flavors in maple syrup. The mechanisms underlying the competitiveness of *Pseudomonas* sp. MSB2019 remain unclear but may involve rapid and efficient growth, production of nutrient-scavenging molecules, superior niche occupation, or direct antagonism via bacteriostatic compounds (Li et al. 2021). It is likely that this strain represents one of the two *Pseudomonas* ASV biomarkers identified in PS-treatment. Shotgun metagenomic sequencing would be necessary to confirm the persistence of inoculated strains throughout the sap flow season and across multiple years (Ventolero et al. 2022).

The bacterial composition of sap differed across the 2 years of study. In 2016, when the tubing of the collection systems was new, sap microbiota richness was lower, as indicated by the Chao1 index (Thukral 2017). However, the overall richness and evenness of bacterial communities was similar across both years. The presence of *Yersinia* spp. in the second year may be explained by the resistance of this genus to IPA (Latham et al. 2022). Disinfection of the tubing with this compound which is still in the collection system at the end of the season and stay until next season may have favored its presence. Modulations of bacterial communities between treatments, sampling times, and years could also be

linked to yeasts and molds, which are integral components of the sap microbiota. Yeast and mold growth patterns showed slight differences between 2016 and 2017. While fungal load reached 4 log CFU/mL sooner in 2016, the final concentration was higher in 2017, reaching 6 log CFU/mL of sap. This value is 2 and 1 log higher than the fungal loads observed by Lagacé et al. (2002, 2015), respectively. It is possible that the microbial load of fungi has been underestimated, as the primary sugar in Dichloran Rose Bengal Chloramphenicol medium is glucose and its composition is not adapted for recovering xerophilic fungi.

According to Historical Climate Data website of Government of Canada (<https://climate.weather.gc.ca/>), the average temperature at the Arthabaska weather station (located 8.9 km from the experiment site) for March–April was -0.12°C in 2016 and -0.06°C in 2017 (Fig. 7). However, when comparing the months individually, March 2016 was warmer than 2017, -2.60 compared to -6.26°C , respectively. In April, the average temperature was warmer of 2°C in 2017 (6.47°C vs. 2.18°C) with two heat peaks reaching 15°C . The higher temperature in April 2017 could be partially explained the higher yeast and mold loads in sap collected later in the 2017 flow season and differences observed in bacterial composition. The higher temperatures in 2017 could also interacted with the fact that the tubing was new in 2016, while it had aged by 2017. Although cleaning the tubing with IPA between flow season reduces the microbial load, it may leave toler-

Table 5. Flavor intensity of maple syrup samples for the different inoculation treatments determined by a sensory panel.

Sensory family	Inoculation*	Sampling period				
		D1	D2	Mu	F1	F2
2016						
Global flavor	<i>P</i> value [†]	0.4219	0.1489	0.9254	0.6990	0.2867
	PS	4.15	3.65	3.65	3.45	4.15
	JL	3.90	3.85	3.90	3.75	4.60
	PF	3.70	4.75	3.75	3.25	4.45
	Control	3.65	3.95	3.75	3.55	3.80
Maple flavor	<i>P</i> value	0.3740	0.8976	0.8541	0.0714	0.0177
	PS	4.65	3.80	3.80	3.05	3.20 ^c
	JL	4.00	4.10	3.75	3.65	3.70 ^{bc}
	PF	4.30	4.15	3.70	2.60	4.60 ^a
	Control	4.00	4.20	4.05	3.50	4.10 ^{ab}
Off-flavor	<i>P</i> value	0.7197	0.4764	0.8429	0.4941	0.2099
	PS	4.35	3.90	4.25	4.25	4.75
	JL	4.10	4.35	4.05	4.00	4.50
	PF	4.40	4.05	4.05	3.90	4.10
	Control	4.10	4.45	4.15	4.15	4.3
2017						
Global flavor	<i>P</i> value	0.5458	0.8301	0.1215	0.0341	0.0139
	PS	4.00	3.90	4.70	4.80 ^a	4.35 ^a
	JL	4.45	4.15	4.15	4.05 ^b	4.15 ^a
	PF	4.05	4.15	4.05	3.95 ^b	3.70 ^{ab}
	Control	3.95	3.95	3.90	4.25 ^{ab}	3.30 ^b
Maple flavor	<i>P</i> value	0.3877	0.5899	0.2855	0.3248	0.0124
	PS	3.95	4.30	4.20	4.55	4.45 ^a
	JL	3.50	3.95	3.55	4.20	4.25 ^{ab}
	PF	3.75	3.85	4.15	3.95	3.60 ^{bc}
	Control	4.10	4.05	4.05	4.00	3.40 ^c
Off-flavor	<i>P</i> value	0.0140	0.8299	0.5665	0.5117	0.2237
	PS	3.35 ^b	4.10	4.25	4.20	4.00
	JL	4.25 ^a	4.25	4.30	3.90	3.65
	PF	4.25 ^a	4.30	4.40	4.15	3.75
	Control	3.90 ^{ab}	4.20	4.05	4.15	4.20

*PS: *Pseudomonas* sp. MSB2019; JL: *Janthinobacterium lividum* 100-P12-9; PF: *Pseudomonas fluorescens* ATCC 17926; Control: non-inoculated.

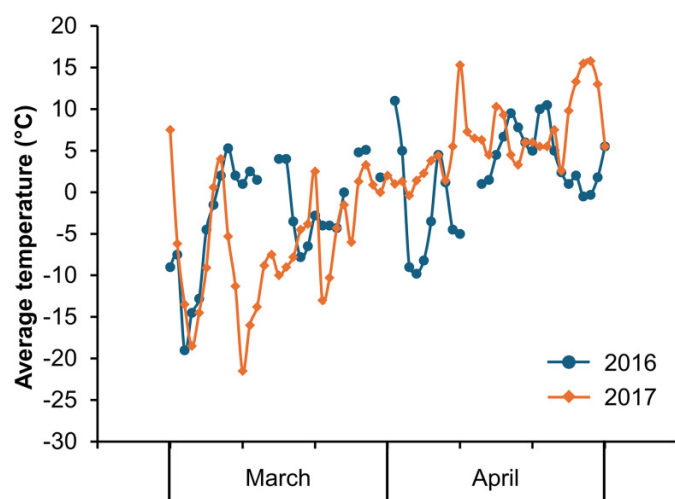
[†]Different letters indicate a significant difference ($P < 0.05$). Probability values less than 0.05 are non-significant and indicate no difference between treatments.

ant microorganisms, as mentioned previously, and biofilm residues on the surface, such as proteins and polysaccharides. It has been shown that even if a surface is sterilized, the presence of biofilm residues can act as nucleation sites stimulating formation of new biofilm upon subsequent contact with biofilm-producing microorganisms (Chmielewski and Frank 2003).

The sampling period had a greater impact on physicochemical characteristics of sap and syrup, regardless inoculation status. The observed decrease in pH of sap is attributed to fermentation of sucrose into reducing sugars by microorganisms which can be fermented further through microbial metabolism (Lagacé et al. 2002). The difference in syrup pH was not observed, likely due to the higher buffering capacity

of the syrup compared to the sap. This fermentation process led to the production of organic acids, such as acetic and lactic acid. In 2017, the microbial growth resulted in higher acetic acid concentration in both sap and maple syrup. While the amount of lactic acid in the sap was below the detection limit, its presence was detectable in the syrup due to the concentration effect during processing. In 2017, the lactic acid concentration in syrup was twice as high as in 2016, while acetic acid levels were seven times higher in 2017. The lactic acid production is commonly due to the presence of lactic acid bacteria, but there abundance were under 0.01% in the present study. Acetic acid is produced by both bacteria, such as *Enterobacteriaceae* and fungi (Solé et al. 2000; Demain and Martens 2017), whose biomass was notably higher in 2017.

Fig. 7. Average temperature in °C recorded by Environment and Climate Change (Government of Canada) between March and April 2016–2017 (<https://climate.weather.gc.ca/>).



Oxalic acid, which was also twice as high in 2017, can be produced by mycorrhizal fungi and wood-degrading fungi. Species such as *Pseudomonas fluorescens* have been reported to produce this organic acid (Palmieri et al. 2019). Shikimic acid was detected only in 2017. This organic acid can be produced naturally by bacteria such as *Escherichia coli* via the shikimic pathway (Krämer et al. 2003). This species was not detected in the present study, but probably other *Gammaproteobacteria* member could have been responsible. Tartaric acid was only detected in 2016, which may be related to the higher biomass observed in the sap during 2017. Molds such as *Penicillium charlesii* and bacteria such as *Pseudomonas* sp. have been identified as having the capacity to metabolize tartaric acid (Dagley and Trudgill 1963; Klatt et al. 1969).

Conclusion

In the present study, inoculating the sap collection tubing systems with *Pseudomonas* sp. MSB2019 seemed to lead to a distinct bacterial composition in the sap compared to the other strains and the non-inoculated control. Notably, *Yersinia* was not observed in sap originated from tubing collection system inoculated with strain MSB2019. Maple syrup produced from this treatment at the end of the 2017 season showed improved organoleptic qualities, particularly in terms of overall and maple flavor. Given the significant differences in sap composition observed between the 2 years, future research should investigate the impact of inoculation over an extended period to assess the potential for long-term colonization. Moreover, as this experiment was conducted in a sugarbush with excellent maple syrup production practices and no prior quality issues, it would be valuable to evaluate the effects of inoculation with *Pseudomonas* sp. MSB2019 in various sugarbushes, including those with known syrup defects related to microbial activity. Before implementing these practices in a commercial maple syrup operation, identification of this strain through whole-genome sequencing would

be advisable and regulatory authorities should be consulted for approval.

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Data availability

Raw sequencing data are available at Bioproject number PR-JNA1160158 at <http://www.ncbi.nlm.nih.gov/bioproject/>. Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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The authors declare there are no competing interests.

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Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjm-2024-0225>.

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