Canadian Journal of Soil Science

OPEN ACCESS | Short communication

Spatial dependency and independency of nitrogen in lowbush blueberry commercial fields

Anthony J. Pelletier[a], **Jean Lafond**[b], **and Maxime C. Paré**[a]

[a]Laboratoire sur les écosystèmes terrestres boréaux (EcoTer), Département des sciences fondamentales, Université du Québec à Chicoutimi, Saguenay, QC G7H 2B1, Canada; [b]Research and Development Centre, Agriculture and Agri-Food Canada, Normandin, QC G8M 4K3, Canada

Corresponding author: **Maxime C. Paré** (email: maxime.pare@uqac.ca)

Abstract

Rhizomes of wild lowbush blueberry (*Vaccinium angustifolium* Aiton) extend horizontally, creating spatial dependency when fertilization trials are performed. Knowing this spatial dependency would help researchers to better design field studies. Here, we used labelled nitrogen (N) fertilizer (^{15}N-$(NH_4)_2SO_4$) to measure N translocation among blueberry stems for one old (56 year) and one younger (15 year) commercial field. Leaf ^{15}N concentrations at the tip-dieback stage were used to monitor N acquisition. No difference between sites suggests no field age effect on N translocation. Spatial dependency and independency were reached for distances of $\leq$0.75 and $\geq$1.75 m from the fertilizer application point, respectively.

Key words: *Vaccinium angustifolium*, *Vaccinium myrtilloides*, nitrogen fertilizer, rhizome translocation

Introduction

Wild lowbush blueberry (*Vaccinium angustifolium* Aiton and *Vaccinium myrtilloides* Michaux) is an ericaceous shrub species that germinates from seeds and spreads underground through extensive rhizomatic networks (Beers et al. 2019). These reproductive and colonization strategies create both high genetic heterogeneity and strong connectivity among clones. Rhizomes are known to be slow-growing organs that colonize the upper organic soil horizons, namely the leaf litter, folic material, and the humic material. The rhizome network connects clones together, which facilitates nutrient translocation among individuals and helps wild blueberry to colonize new soil areas. The translocation of nutrients through this rhizomatic network may create strong spatial dependency between plants in commercial fields.

Fertilization trials are often performed under managed field conditions since fertilizers such as N considerably improve fruit yields (Penney and McRae 2000). In most of them, study plots are separated by buffer distances to limit underground rhizome connections. On small scales ($\leq$1 m^2), polyethylene sheets can be inserted into the soil profile to a depth of 30–40 cm to physically isolate rhizomes (Marty et al. 2019). On larger scales, buffer distances between plots have encompassed 1 m (Penney and McRae 2000), 1.5 m (Lafond 2009), and 3 m (Fournier et al. 2020), with no clear consensus on which distance should be used. Moreover, there is no mention of an inner plot buffer zone (a no-sample strip inside the plot to minimize contamination from outside the plot) in the literature. Since wild blueberry can extend their rhizomes over several metres over time (Morin 2008), it remains unknown whether a buffer strip of 1 m within research plots is sufficient to maintain spatial independency among the plots.

The main objective of this study was to evaluate the distance at which N spatial independency is reached. More particularly, the study evaluates the N translocation distances in an old and a younger lowbush blueberry field using labelled N fertilizer. We hypothesized that the spatial dependency distances would differ between sites, with the older field offering more mobility for the labelled N due to its extended and well-established rhizome network as compared with the younger site.

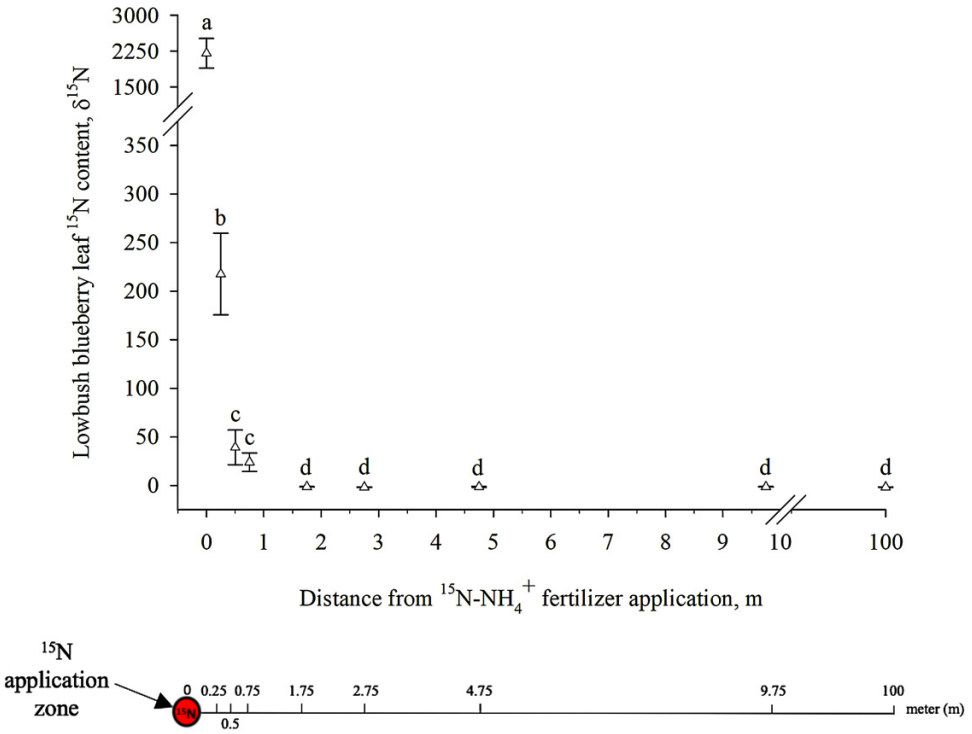
Materials and methods

Experimental set-up

The experiment was conducted in two wild lowbush blueberry (*V. angustifolium* and *V. myrtilloides*) commercial fields in June 2020, in Normandin, Lac Saint-Jean, QC, Canada. Site 1 (48°46′73″N, 72°33′91″W) was a blueberry field established in 1964 (56 years old), whereas site 2 (48°50′67″N, 72°39′57″W) was established in 2005 (15 years old).

A complete randomized block design experiment was established at both sites with the site and the distance from the labelled fertilizer application point as main fixed factors. The distance factor corresponded to nine leaf sampling distances from 0 to 100 m from the fertilizer application point (see below). At each site, ^{15}N labelling and sampling were done in five blocks or replicates with a distance of at least 100 m from each other to ensure their spatial

Fig. 1. Lowbush blueberry leaf ^{15}N content ($\delta^{15}N$) according to distance from ^{15}N-NH_4^+ fertilizer application point (m) for both sites combined ($n = 10$). Values are mean ± standard error for non-transformed data. Values not sharing the same letters are significantly different at $P < 0.05$.



independency. For each block, 5.6 g of labelled N ($(NH_4)_2SO_4$, 5% ^{15}N (Sigma–Aldrich Inc.)) was diluted in 300 mL of water and then a single application was done with a manual garden pressure sprayer (Rampro, Nova Scotia, Canada) within a 50 cm diameter circular application template. This amount of labelled N represents an equivalent of 60 kg of N ha^{-1}. The labelled fertilizer applications were done in spring (3 June 2020), on a stem-free soil (beginning of the pruning year). A 46 cm long wooden stick was installed in the middle of the application circle to guide leaf sampling. Seven days after the beginning of the experiment, the whole fields received 30 and 50 kg of N ha^{-1} on 10 June 2020, at sites 1 and 2, respectively. This second fertilization was made by the growers, with non-labelled nitrogen sulfate completed with phosphorus (P) as triple superphosphate and potassium (K) sulfate to improve plant growth and fruit yields. For site 1, 200 kg of fertilizer was applied per hectare following a N–P–K ratio of 15:10:10 as site 2 received 300 kg ha^{-1} with a 16.8:6.7:6.7 ratio.

Sample collection and analysis

Wild blueberry leaves were sampled for foliar analysis at the tip-dieback stage (third week of July 2020), as this sampling time is well established in the industry to monitor plant nutrient status (Lafond 2009). For each block or replicate, a 100 m marked rope was attached to the wooden stick to guide leaf sampling at nine pre-established sampling distances relative to the centre; 0, 0.25, 0.50, 0.75, 1.75, 2.75, 4.75, 9.75, and 100 m outside the application zone. A 360° rotation was done around the central wooden stick to sample leaves at the corresponding distance. Leaves from about 15 stems for each distance and replicate were randomly selected indiscriminately of their age and their position on the stems. The sampled leaves were placed in paper bags and then dried at 60 °C for 72 h. One subsample (i.e., ~10 g of dry weight) was randomly taken from each bag, put into a 20 mL scintillation vial, and independently ground to powder using stainless steel balls and rotatory tumbler (75RT, Diamond Pacific, Barstow, CA, USA). About 3.8 (±0.5) mg of ground leaf material was then wrapped in tin capsules and sent to the Centre de recherche en géochimie et géodynamique (UQÀM, Montréal, QC, Canada) for ^{15}N analyses. Nitrogen isotopic ratios were measured using an elemental analyzer in continuous flow mode, coupled to an isotope ratio mass spectrometer (Micromass Isoprime 100, Cheadle, UK). Values of $\delta^{15}N$ are expressed in ‰ versus air (±0.2‰ at 1σ).

Statistical analysis

A linear mixed model (mixed model procedure) was used for variance analysis with the distance and site as fixed factors, and the block as a random factor. Data were $\log_{10}$-transformed to improve normality and homoscedasticity. Tukey's post-hoc test was used with a significant level of $\alpha = 0.05$ to compare significance among sampling distances from fertilizer application point. All statistical analyses were performed with SPSS software, version 24.

Results and discussion

A distance effect was found ($F = 56.68$; df = 8; $P < 0.001$), whereas the site ($F = 0.10$; df = 1; $P = 0.758$) and the site × distance interaction term effects ($F = 1.18$; df = 8;

$P = 0.324$) were not statistically significant. This lack of significance among sites suggests that the age of these sites has no effect on N translocation distances. However, these results should be interpreted cautiously since the site—or—age was not fully replicated with more sites. The leaf ^{15}N concentrations strongly decreased from 0 to 0.75 m (Fig. 1). Moreover, no significant difference in leaf ^{15}N concentration was found between 1.75 and 100 m (Fig. 1), which suggests that leaf ^{15}N concentrations reached independence (i.e., natural ^{15}N abundance) at or after 1.75 m. Therefore, our results clearly show that spatial dependency was significant for distances of 0.75 m and less from the N fertilizer application point. Inversely, our results show that complete spatial independency was obtained at a distance of between 0.75 and 1.75 m from the fertilizer application point. Nevertheless, we suggest 1.75 m as a conservative minimal distance to meet spatial independency in wild blueberry commercial fields.

Even though we did not directly quantify N translocation within plants, rhizome structures remain the most probable explanation for this N spatial dependency. These results support our hypothesis that labelled N is transferred spatially within the field, up to a distance of between 0.75 and 1.75 m from the application point. To ensure spatial independency between research plots when N fertilizer trials are performed, buffer distances of 1.75 m outside and inside the plots should be used to avoid potential contamination. If not, some concerns might appear in the conclusions since independence of observations is an important assumption for many statistical models and analyses. However, it is important to keep in mind that conclusions from studies that used 1 m as buffer distance are not necessarily wrong. In fact, despite N still significantly translocating at 0.75 m from the application point (Fig. 1), the great majority of leaf N was not derived from the fertilizer. Indeed, based on our ^{15}N values (Fig. 1), approximately 99% of the N at 0.75 m was not derived from the fertilizer. Nitrogen recovery can also be influenced by fertilizer's solubility. Ammonium sulfate dissolves in the soil solution and can therefore be absorbed by plants away from the application zone. Nevertheless, we suggest 1.75 m as a conservative distance to maintain N spatial independency when fertilizer trials are performed in wild blueberry commercial fields.

Acknowledgements

The authors thank the Syndicat des Producteurs de Bleuets du Québec (SPBQ) and the Natural Sciences and Engineering Research Council of Canada (NSERC) (grant RDCPJ-503182-16) for their financial support. The authors would also like to acknowledge Jean-Marc Bernard (Bleuetière Coopérative de Normandin) and Joël Lacasse (Corporation d'Aménagement Forêt Normandin) for providing access to their blueberry fields. Authors also thank Dr. Charles Marty for reviewing and editing the final version of the manuscript.

Article information

History dates

Received: 27 April 2022
Accepted: 7 September 2022
Accepted manuscript online: 15 September 2022
Version of record online: 3 February 2023

Copyright

Data availability

Primary research data are available on demand at anthony.pelletier1@uqac.ca.

Author information

Author ORCIDs

Anthony J. Pelletier https://orcid.org/0000-0002-3658-9350
Maxime C. Paré https://orcid.org/0000-0003-3482-4828

Author contribution

Conceptualization: MCP
Data curation: AJP
Formal analysis: AJP
Funding acquisition: MCP
Investigation: AJP
Methodology: AJP
Project administration: MCP
Supervision: JL, MCP
Validation: JL, MCP
Writing – original draft: AJP
Writing – review & editing: AJP, JL, MCP

Competing interests

The authors have no conflict of interest to disclose.

References

Beers, L., Rowland, L.J., and Drummond, F. 2019. Genetic diversity of lowbush blueberry throughout the United States in managed and non-managed populations. Agriculture, **9**: 113. doi:10.3390/agriculture9060113.

Fournier, M.-P., Paré, M.C., Buttò, V., Delagrange, S., Lafond, J., and Deslauriers, A. 2020. How plant allometry influences bud phenology and fruit yield in two *Vaccinium* species. Ann. Bot. (London), **126**: 825–835. doi:10.1093/aob/mcaa083.

Lafond, J. 2009. Optimum leaf nutrient concentrations of wild lowbush blueberry in Quebec. Can. J. Plant Sci. **89**: 341–347. doi:10.4141/cjps08142.

Marty, C., Lévesque, J.-A., Bradley, R.L., Lafond, J., and Paré, M.C. 2019. Contrasting impacts of two weed species on lowbush blueberry fertilizer nitrogen uptake in a commercial field. PLoS ONE, **14**: e0215253. doi:10.1371/journal.pone.0215253.

Morin, C. 2008. Étude morphologique et physiologique du rhizome du bleuet nain: une contribution à l'amélioration de la régie de culture. M.Sc. thesis. Université Laval, Quebec City, QC. [in French].

Penney, B., and McRae, K. 2000. Herbicidal weed control and crop-year NPK fertilization improves lowbush blueberry (*Vaccinium angustifolium* Ait.) production. Can. J. Plant Sci. **80**: 351–361. doi:10.4141/P99-080.