

1 **SPECIES-SPECIFIC RESPONSES TO FOREST SOIL INOCULUM IN**
2 **PLANTED TREES IN AN ABANDONED AGRICULTURAL FIELD**

3
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1 **Highlights**

- 2 • Tree seedlings planted in an abandoned agricultural field responded differently to forest soil
3 inoculum according to tree species.
- 4 • The arbuscular mycorrhizal tree species red ash and red maple responded weakly to forest
5 soil inoculation.
- 6 • Conspecific inoculum had negative effects on the growth of the ectomycorrhizal (EM) tree
7 species red oak.
- 8 • Positive effects were observed on the growth of yellow birch seedlings.

10 **Abstract**

11 Tree plantations are commonly used to restore abandoned agricultural fields with varying degrees of
12 success. Agricultural soils differ from forest soils in nutrient availability and microbial communities.
13 The objective of this study was to test the effect of adding small amounts of forest soil on the survival,
14 growth and rates of mycorrhizal fungal colonization of trees planted in an abandoned agricultural
15 field over the crucial first three growing seasons. Seedlings of two arbuscular mycorrhizal (AM) and
16 two ectomycorrhizal (EM) tree species were planted in an abandoned agricultural field. Soil inocula
17 were taken from four forest stands, each dominated by one of the planted species. Half of the soil
18 samples were sterilized before inoculation to distinguish microbial from nutrient effects. The effect
19 of the quantity of soil inoculum added was tested using 300 and 1500 ml of forest soil. Tree mortality
20 was low and did not vary between treatments. The growth of EM tree species responded, positively
21 or negatively, to forest soil inoculation. A negative feedback was detected on the growth of red oak
22 seedlings inoculated with red oak soil. Seedlings inoculated with EM sterilized soils were smaller
23 than control seedlings, presumably due to lower nutrient availability of EM forest soils compared to
24 agricultural field soil. The majority of the effects, either positive or negative, were observed the first
25 year. After three seasons of growth, only yellow birch seedlings that had received 1500 ml of non-
26 sterilized red oak soil still benefited from soil inoculation. More research is needed in nutrient-limited

1 soils to determine whether inoculation would have greater or longer term benefits on tree survival
2 and growth.

3

4 **Keywords** Abandoned agricultural field; ecological restoration; forest soil inoculation; tree
5 seedlings; tree growth; mycorrhizae.

6

7 **1. Introduction**

8 Long-term intensive agricultural activities may decrease soil organic carbon, nutrient availability as
9 well as microbial biomass (Dick, 1992; Lal, 2004; Rosenzweig et al., 2016). A reduction in
10 mycorrhizal diversity and mycelium abundance has also been reported in such sites (Jonhson, 1993;
11 Helgason et al., 1998; Alguacil et al., 2008). It has recently been suggested that soil microbial
12 communities could be manipulated to enhance the success of ecological restoration (Heneghan et al.,
13 2008; Harris, 2009; Hoeksema et al., 2010). Mycorrhizal fungal and bacterial inoculations have been
14 previously tested to improve the survival and growth of outplanted nursery-produced tree seedlings
15 (Trappe, 1977; Kropp and Langlois, 1990; Torrey, 1992). However, plant response (e.g. biomass) to
16 inoculation could be greater if instead of using a single mycorrhizal fungus, several mycorrhizal
17 fungal species and non-mycorrhizal microbes are present in the inoculum, or whole–community soil
18 is used as an inoculum (Hoeksema et al., 2010; Urgiles et al., 2014). In effect, mycorrhizal function
19 and behaviour are generally stimulated by an array of soil organisms, although some inhibitory
20 interactions are possible (Fitter and Garbaye, 1994).

21

1 A simple method to inoculate trees would be to add soil containing desirable mycorrhizal fungal
2 spores to a site that is deficient in these fungi (Schwartz et al., 2006), such as forest vs agricultural
3 soils. Diversities of arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi are higher in forest
4 soils than in agricultural soils, but AM fungi are more abundant than EM fungi in agricultural soils
5 (Helgason et al., 1998; Berman and Bledsoe, 1998; Dickie and Reich, 2005). Moreover, soils with
6 high carbon levels and well-balanced nutrients generally have a positive impact on tree nutrition
7 (Pinno et al., 2010; Ens et al., 2013). Therefore, adding a small amount of forest soil to an abandoned
8 agricultural field could potentially create planting microsites that optimize tree survival and growth.

9

10 Most studies exploring the effects of forest soil inoculum on tree seedlings have used pot studies
11 and/or have conducted short-term (< 1 year) experiments (Packer and Clay, 2000; O'Brien et al.,
12 2011; Urgiles et al., 2014; Dulmer et al., 2014). In pot studies, effects of forest soil transfer were
13 observed on seedling growth, but the soil inoculum was mixed with a sterilized substrate (Borchers
14 and Perry, 1990; O'Brien, Gomola and Horton, 2011; Urgiles et al., 2014). Thus, tree seedlings had
15 access to an environment in which the added microbes were not competing with field microbes.
16 Previous field experiments using soil transfer to tree seedlings usually showed an increase in EM
17 fungal colonization, but the presence or absence of effects on growth depended on soil provenance,
18 field conditions, and tree species (Amaranthus and Perry, 1987; Helm and Carling, 1993; Berman and
19 Bledsoe, 1998; Dickie et al., 2007).

20

21 The main objective of this study was to evaluate the effects of adding forest soil to tree seedlings
22 planted in an abandoned agricultural field and to follow their survival and growth over three growing
23 seasons. The specific objective was to compare the responses of two ectomycorrhizal (EM) tree

1 species and two arbuscular mycorrhizal (AM) tree species to different soil inoculation treatments.
2 Since associations with EM fungi usually confer more benefits to tree seedlings than associations
3 with AM fungi (van der Heijden and Horton, 2009; Bradford, 2014) and AM fungi should be more
4 abundant than EM fungi in agricultural soils, a greater response from EM tree seedlings was expected.
5 We also questioned the effect of host specificity and soil provenance: does soil inoculum collected
6 under a mature tree of the same species have effects similar to that of inoculum collected under a
7 different species on the receiving seedling? Since many mycorrhizal fungi are not host specific (van
8 der Heijden and Horton, 2009), we hypothesised no effect of soil provenance.

9

10 **2. Materials and methods**

11 *2.1 Study area*

12 The experiment was conducted at the city of Montréal's tree nursery, in the suburb of L'Assomption
13 (45°48'38"N; 73°26'26"W). The region is characterized by a humid continental climate. For the 1981-
14 2010 period, the average annual temperature recorded at the nearest weather station (Verchères,
15 45°46'N; 73°22'W) is 6.6°C, with monthly means of 21°C in July and -10°C in January while average
16 annual precipitation is 984 mm, of which almost 20% falls as snow (Environment Canada, 2015).
17 The experiment was established on two adjacent abandoned agricultural fields separated by a 5 m
18 wide buffer of young Norway spruce (*Picea abies*). Soil is a fine to very fine sandy loam (IRDA,
19 2008). Fields were mown once or twice a year since the end of agricultural crop production more than
20 15 years ago. Mowing promoted ruderal herbaceous vegetation dominated by grass and clover species
21 which are hosts of arbuscular mycorrhizal species.

22

1 2.2 Experimental design

2 This controlled field experiment began in May 2012 and the last growth measurements were taken at
3 the end of the third growing season in August 2014. Four 12 × 36 m blocks were delineated in the
4 largest abandoned field. Another block of the same size was delineated in the smallest abandoned
5 field. Blocks were located at least 5 m away from a forested strip and more than 30 m from the road
6 to avoid edge effects. Each block was divided into two sections: the first for AM tree species and the
7 second for EM tree species. Two AM tree species, red ash (*Fraxinus pennsylvanica* Marshall) and
8 red maple (*Acer rubrum* L.), and two EM tree species, yellow birch (*Betula alleghaniensis* Britton)
9 and northern red oak (*Quercus rubra* L.), were planted. Each half block was divided into 9 plots using
10 a random split-plot design. For the first half of each block, the treatments were: (1) soil quantities (0
11 ml, 300 ml or 1500 ml of loose soil); (2) soil sterilization (sterilized or not, hereinafter named live
12 soil); (3) soil provenance (red ash or red maple forest soils) and (4) tree species (red ash or red maple)
13 (Fig. 1a). For the other half of the blocks, the first two factors were the same, but the soil provenance
14 and tree species treatments were replaced by the EM species yellow birch and red oak (Fig. 1b). In
15 each plot, 3 seedlings of the same species (9 plots, 5 blocks, 4 species) were planted (total of 540
16 seedlings).

17

18 These four hardwood species were selected because they are native to the area and seedlings are
19 readily available. All four species have intermediate shade tolerance (Niinemets & Valladeres 2006).
20 Container-produced one year-old seedlings were provided by the Berthierville nursery of the Quebec
21 Ministry of Forests, Wildlife and Parks. This nursery does not inoculate seedlings with any microbes.
22 Once delivered, seedlings were kept in a dark room (4°C, 90% humidity) before planting. Seedlings

1 were manually planted at 2 m spacing on May 14 and 15, 2012 after the herbaceous vegetation was
2 mowed.

3

4 Following planting, a white 50 × 50 cm polyester and polypropylene felt mulch (Arbo-Pro, Texel,
5 Saint-Elzéar-de-Beauce, Canada) was placed around each tree seedling to reduce herb competition
6 and maintain a stable climate while allowing water to penetrate the soil. In the rows between
7 seedlings, vegetation was mowed every month during the summer. Each tree seedling was protected
8 from small mammal predation using a plastic tree protector (Timm Enterprises Ltd, Milton, Canada).
9 Tree protectors were 30 cm high for red ash, red maple and red oak seedlings, and 20 cm for the
10 smaller yellow birch seedlings. No signs of herbivory were observed.

11

12 *2.3 Soil collection and inoculation*

13 Soils were collected in forest stands located within a 100 km radius of the study area between May 7
14 and 9, 2012. AM forest soil samples were collected in the forests in proximity to the island of
15 Montréal, whereas EM forest soils were collected in the Lower Laurentians, starting about 60 km
16 north of Montréal where the EM species are more abundant. More specifically, soil supporting red
17 ash trees was collected in a woodlot outside the city of Laval, in an agricultural setting, approximately
18 35 km southwest of the study area (45°40'36"N; 73°43'39"W). The woodlot is composed of many
19 broadleaf species, but the soil was taken in a section dominated by more than 70% mature red ash
20 trees. The soil has a high activity of earthworms (A. St-Denis, pers. obs.) due to its neutral pH
21 (calcareous glacial till). It developed into a Melanic Brunisol (Soil Classification Working Group,
22 1998) or Eutric Cambisol (IUSS Working Group WRB, 2015).

23

1 Soil supporting red maple trees was collected in a relatively pure red maple stand bordering a river,
2 near the city of Boisbriand, located about 50 km southwest of the study area (45°35'52"N;
3 73°50'04"W). The soil developed from a fluvial deposit and transitions from Gleysols to Gray Brown
4 Luvisol (or Albic Luvisol) (Soil Classification Working Group, 1998; IUSS Working Group WRB,
5 2015), depending on drainage. Soil has high P content due to previous agricultural land use on the
6 uphill portion of the site.

7

8 Soils supporting yellow birch and red oak were collected at the *Station de biologie des Laurentides*
9 of the *Université de Montréal*, in Saint-Hippolyte, 95 km northwest of the study area. The
10 Precambrian Shield from which the yellow birch and red oak soils were sampled is characterized by
11 thin glacial till soils derived from felsic (acidic) rocks (Bélangier et al., 2012) that generally develop
12 into Orthic Humo-Ferric Podzols (Soil Classification Working Group, 1998, or Orthic Podzols, IUSS
13 Working Group WRB, 2015). For yellow birch, soil was sampled in a sugar maple — yellow birch
14 stand (45°58'51.3"N; 74°00'52.7"W) located on a gentle slope. For red oak, soil was collected in a
15 sugar maple — red oak stand (45°58'17.0"N; 73°59'53.4"W) located on a hilltop. In these two stands,
16 sugar maple was the dominant species while yellow birch was the co-dominant species in the first
17 one and red oak, in the other.

18

19 As microbe species and abundance could change during the growing season, another soil collection
20 was conducted on August 16, 2012, at the same sites, but in areas undisturbed by the previous soil
21 collection. In each forest type, soil samples were collected from under at least six mature trees (stems
22 with DBH > 20 cm) of the target species in an area where the other tested species of the same
23 mycorrhizal association was not present. Coarse woody debris and surface litter were removed before

1 collecting soil (0-25 cm depth) between 0.5 and 1 m from a mature tree. To avoid contamination,
2 collection tools were washed with a bleach solution (10% of sodium hypochlorite) and rinsed with
3 water between each forest soil collection. All soil samples from a given forest site were combined
4 into a single bulk sample. Bulk samples were sieved using a 1 cm mesh, but fine root segments were
5 kept. Half of the bulk samples were immediately stored in a cool dark room (4°C, 90% of humidity)
6 for a few days before being brought to the planting site. The other half of the bulk samples received
7 a gamma irradiation treatment at the Nordion's Gamma Centre (Laval, Québec) to eliminate
8 invertebrates, fungi and other microorganisms (McNamara et al. 2003). The first batch of soils was
9 sterilised May 10 to 14, 2012, with a minimal dose of 51.1 kGy and a maximal dose of 73.9 kGy. The
10 second batch was sterilised August 17 to 21, 2012, with a minimal dose of 54.7 kGy and a maximal
11 dose of 80.5 kGy.

12

13 Inocula of 100 ml or 500 ml of live or sterilized soils were added in three steps for a total of 300 ml
14 or 1500 ml of soil added. The first soil inoculum (100 or 500 ml) was placed in the planting hole just
15 before the seedling was planted and the second was applied at the soil surface around the seedling
16 ($\approx 300 \text{ cm}^2$) immediately after planting. The third application was also at the soil surface around the
17 seedling but three months after planting (August 16 and 21, 2012). In each block, the controls (three
18 individuals per species) did not receive any soil inoculum.

19

20 *2.4 Soil analyses*

21 Subsamples of each soil type were air-dried and sieved at 2 mm before determining sand, silt and clay
22 fractions using the hydrometer method Done (Kroestch and Wang, 2008) and soil pH in water
23 (soil:solution ratio of 1:2.5). Organic C, total N and S were determined on finely ground samples by

1 combustion and infrared detection (CNS 2000, LECO Corporation, MI). Phosphorus, K, Ca, Mg, Mn,
2 Al, Fe and Na were extracted from these samples using an unbuffered Mehlich III solution (Ziadi and
3 Sen Tren, 2007) and measured by inductively coupled plasma emission spectroscopy (Optima
4 7300DV, PerkinElmer, MA). Effective cation exchange capacity (CEC) was operationally defined as
5 the sum of Ca, Mg, K, Na, Al, Fe and Mn, whereas base saturation was expressed as the sum of Ca,
6 Mg, K and Na on CEC (Hendershot et al., 2007).

7

8 *2.5 Data collection*

9 Survival, height and diameter at 5 cm above the ground were measured three weeks after planting
10 (June 4, 2012), at the end of the first (September 19, 2012) and second (October 4, 2013) growing
11 seasons, and on August 5, 2014. On September 24 2014, 15 seedlings per species (1 seedling per
12 block (5 blocks) in plots where 1500 ml of live soil collected in one forest was inoculated, 1 seedling
13 per block in plots where 1500 ml of live soil from the second forest was inoculated and 1 seedling
14 per block the control plots) were randomly excavated using a side-digger which can excavate 60 cm
15 wide and 50 cm deep. Roots were stored in a freezer at -14°C until analysed.

16

17 Roots were gently washed under tap water and examined for mycorrhizal fungal colonization. For
18 AM tree species, 25–30 root tips per seedling (approximately 1.5 cm long) were randomly selected.
19 The ink and vinegar staining technique developed by Vierheilig et al. (1998) was adapted to clear and
20 stain red ash and red maple roots. Roots were cleared by soaking them in 10% KOH at 90°C for 10
21 minutes for red ash roots and 3 hours for coriaceous red maple roots. After being rinsed with tap
22 water, they were bleached with 3% H_2O_2 for 10 and 60 minutes for red ash and red maple roots,
23 respectively. Roots were acidified in vinegar (5% acetic acid) for 10 and 60 minutes for red ash and

1 red maple roots. They were soaked in a solution of 5% black ink (Sheaffer Skrip #94231, Sheaffer
2 Manufacturing Co., Fort Madison, IA) and 95% vinegar overnight at room temperature. Roots were
3 rinsed several times with tap water acidified with a few drops of acetic acid. They were then placed
4 in a destain solution made of 50% glycerol, 45% distilled water and 5% of HCl (1%) solution for one
5 week. Arbuscular colonization (AC) and hyphae colonization (HC) were assessed following
6 McGonicle et al. (1990). Arbuscules are produced by mycorrhizal fungi while hyphae may be
7 produced by both mycorrhizal and non-mycorrhizal fungi (McGonicle et al., 1990). Each of the 25 to
8 30 root tips per seedling were examined under a compound microscope (200× magnification) at three
9 locations, for a total of 75 to 90 locations examined per sample.

10

11 For EM tree species, approximately one hundred 1 cm root tips were randomly selected among all
12 roots collected per excavated seedling and examined under a dissecting microscope (10-50×). EM
13 fungal colonization for red oak and yellow birch seedlings was assessed as the proportion of root tips
14 with mycorrhizal structures (identified by color, branching, shape, texture and presence of emanating
15 hyphae) over the total number of root tips evaluated. A subset of 15–25 root tips/seedling was
16 observed under a compound microscope (100–400×) to confirm the presence of a Hartig net for EM
17 tree species.

18

19 *2.6 Statistical analyses*

20 Chi-square tests were used to assess seedling survival. Analyses of variance for repeated
21 measurements (ANOVAR) and univariate analyses of variance (ANOVA) were performed for each
22 species to evaluate the effects of soil inoculum quantity, soil sterilization, soil provenance, time and
23 their interactions on seedling height and diameter. Analyses were performed following a split-plot

1 design (3×2 factorial model): Block (random) × Soil inoculum quantity × Soil sterilization × Soil
2 provenance. The data satisfied the assumptions of homoscedasticity and normality. Student's t-test
3 and Tukey tests were used as *post-hoc* tests. The statistical design was not balanced if the control
4 plots (no soil added) were included in the analyses of variance. We thus used a 9×1 factorial model
5 which includes 8 soil treatment combinations (for example: “300 ml of sterilized yellow birch soil”)
6 + the control. To correct for multiple comparisons, the Dunnett method was used as a *post-hoc* test
7 to evaluate whether treatment means were significantly different from control means. Levels of
8 mycorrhizal fungal colonization were compared using ANOVAs and correlated to final tree height
9 and diameter with Pearson correlations. All statistical analyses were performed using JMP 10.0.
10 Statistical significance was determined at $p = 0.05$.

11

12 **3. Results**

13 *3.1 Soil characteristics*

14 Basic physical and chemical characteristics of forest and agricultural field soils are presented in Table
15 1. On the one hand, very high and high levels of extractable (Mehlich III) P were measured for the
16 agricultural soil (68 mg kg⁻¹) and for red maple soil (40 mg kg⁻¹), respectively, compared to the other
17 forest soil types (ranging from 6 to 23 mg kg⁻¹). On the other hand, the agricultural field soil had the
18 lowest organic C (1.44%) and total N (0.13%) levels and some of the lowest exchangeable Ca (5.12
19 cmol₍₊₎ kg⁻¹), CEC (31.4 cmol₍₊₎ kg⁻¹) and base saturation (19.8%) values in comparison to forest soils
20 (2.93-13.0% for organic C; 0.32-0.64% for total N; 1.18-29.9 cmol₍₊₎ kg⁻¹ for exchangeable Ca; 10.5-
21 44.4 cmol₍₊₎ kg⁻¹ for CEC and 3.91-73.0% for base saturation). The molar N (total): P (Mehlich) ratio
22 of the agricultural field soil (42.2) was much lower than all other forest soils (174-2257) (results not
23 shown). The red ash and red maple soils showed higher exchangeable Ca, base saturation and pH

1 than the yellow birch and red oak soils. Soils collected under mature red ash trees had the highest
2 exchangeable Ca (28-30 $\text{cmol}_{(+)}$ kg^{-1}), base saturation (71-73%) and pH (7.25). The red ash and red
3 maple soils had relatively high silt and clay content, whereas the more acidic yellow birch and red
4 oak soils had coarser texture (i.e. sandy loam).

5

6 *3.2 Survival*

7 Seedling survival was high, with no mortality for red ash seedlings, and one red maple and five red
8 oak seedlings that died. Mortality was too low for chi-square tests, except for the EM species yellow
9 birch (22 dead seedlings). However, neither soil inoculum quantity ($\chi^2 = 0.374$; $p = 0.5410$),
10 sterilization ($\chi^2 = 1.016$; $p = 0.3135$) nor soil provenance treatment ($\chi^2 = 1.330$; $p = 0.2488$) affected
11 survival.

12

13 *3.3 Effects of soil treatments on AM tree species growth*

14 AM tree species did not show consistent or persistent responses to the addition of forest soil. None
15 of the factors tested affected red ash height and diameter (Tables A.1–A.2, Fig. A.1). However, after
16 the first growing season (in the fall of 2012), the diameter of red ash seedlings receiving 1500 ml of
17 live red maple soil ($p = 0.01$) and 300 or 1500 ml of sterilized red ash soil ($p = 0.04$ and $p = 0.01$,
18 respectively) was larger than the diameter of seedlings that did not receive forest soil (Fig. 2a). These
19 effects did not persist with time ($F = 0.74$, $p = 0.74$; Table A.1).

20

21 The growth of red maple seedlings inoculated with forest soil was similar to the growth of seedlings
22 in the control (Fig. A.2). However, a significant “soil inoculum quantity” \times “soil sterilization” \times “soil

1 provenance” interaction ($F = 10.99$, $p = 0.001$) was detected for red maple height at the end of the
2 first growing season (Table A.2). Red maple seedlings inoculated with 1500 ml of live red ash soil
3 were taller than seedlings receiving 300 ml, and taller than those inoculated with 1500 ml of live red
4 maple soil. Conversely, red maple seedlings inoculated with soil collected under conspecific trees
5 were taller than those inoculated with red ash soil, but only when 300 ml of live soil was added. These
6 contrasting results do not provide strong evidence for positive effects of forest soil inoculation on red
7 maple seedlings.

8

9 *3.4 Effects of soil treatments on EM tree species growth*

10 The EM birch seedlings inoculated with sterilized soil (1500 ml of yellow birch soil, 300 or 1500 ml
11 of red oak soil) were smaller than control seedlings at the end of the first growing season (Fig. 2b).
12 Furthermore, yellow birch seedlings inoculated with live soil were taller and had a larger diameter
13 during the first year ($F = 32.49$, $p = 0.005$ and $F = 18.20$, $p = 0.01$, respectively) than those inoculated
14 with sterilized soil for all soil quantities and types combined (Fig. 3; Table A.2), but they were not
15 taller than control seedlings (Fig 2b). The effect on height was greater when 1500 ml of soil was
16 added vs 300 ml ($F = 13.55$, $p = 0.02$; Table A.2). In contrast to height, yellow birch seedlings
17 inoculated with yellow birch soil (sterilized or not) had a larger diameter when 300 ml of soil was
18 added instead of 1500 ml ($F = 90.21$, $p = 0.002$; Table A.2). This was only observed after one growing
19 season. By the end of the third season, results had reversed such that diameter growth of birch
20 seedlings inoculated with 1500 ml of live soil was greater than with 300 ml ($F = 14.49$; $p = 0.02$).
21 Moreover, after the third year of growth, seedlings receiving 1500 ml of live red oak soil had a larger
22 diameter than controls ($p = 0.005$; Fig. 2c). These effects coupled with the first year effects suggest
23 some benefits of adding live red oak soil to yellow birch seedlings when planting this species in an
24 abandoned agricultural field.

1

2 Positive and negative feedbacks were observed on the growth of red oak seedlings. Oak seedlings
3 inoculated with live (1500 ml) or sterilized red oak soil (300 or 1500 ml) were smaller than control
4 seedlings after the first and second growing seasons (Fig. 2d,e). As with the other EM species, red
5 oak seedlings were taller when inoculated with live soil vs sterilized soil after the first year ($F = 11.31$,
6 $p = 0.03$; Fig. 4a; Table A.2). Also, they were tallest when a small quantity of soil was added ($F =$
7 27.77 , $p = 0.006$; Fig. 4b). First year red oak seedlings inoculated with red oak soil were smaller than
8 those inoculated with yellow birch soil ($F = 27.29$, $p = 0.006$; Fig. 4c). At the end of the third summer,
9 red oak seedling diameters were larger when inoculated with 1500 ml of soil instead of 300 ml ($F =$
10 11.21 , $p = 0.03$). In contrast to height growth, diameter growth did not differ between the soil
11 treatments and the control (Fig. A.4). In summary, for red oak seedlings, we observed: (1) a positive
12 effect of adding live compared to sterilized soil; (2) a positive effect of adding yellow birch soil
13 compared to red oak soil (although there were no significant benefits of adding forest soil compared
14 to the control) (Fig. A.4); and finally (3) a negative effect of red oak soil on red oak seedling height.

15

16 *3.5 Mycorrhizal fungal colonization*

17 We observed >75% hyphae colonization (HC) for ash and maple seedlings. Forest soil inoculation
18 did not increase arbuscular colonization (AC) of red ash (34 to 51%; $F = 1.09$, $p = 0.38$) and red
19 maple seedlings (68 to 70%; $F = 1.89$, $p = 0.95$) nor the EM fungal colonization of yellow birch (40
20 to 60%; $F = 0.94$, $p = 0.43$) or red oak seedlings (30 to 43%; $F = 2.02$, $p = 0.19$). Hypha of EM fungi
21 observed on EM tree roots were less than 5 cm long. Mycorrhizal fungal colonization was not
22 correlated to tree height ($p > 0.19$) and diameter ($p > 0.22$).

23

1

2 4. Discussion

3 4.1 AM vs. EM tree species response

4 We hypothesized a greater response from EM tree seedlings than AM tree seedlings and no effect of
5 soil provenance because many mycorrhizal fungi are not host specific (van der Heijden and Horton,
6 2009) and because AM fungi should be more abundant than EM fungi due to the presence of grasses
7 in the abandoned old field that we studied. As expected, forest soil inoculum had more effects on EM
8 than on AM tree species. On the one hand, only few temporary, weak or divergent effects were
9 observed for the growth of both AM tree species. On the other hand, yellow birch and red oak
10 seedlings responded positively to the inoculation of live soil compared to sterilized soil in the first
11 growing season, suggesting a possible microbial feedback. However, those first year seedlings did
12 not have better growth than control seedlings. Only birch seedlings receiving 1500 ml of live soil
13 collected under the trees of the other EM species (red oak) had a greater diameter than control
14 seedlings after three growing seasons. This effect could be driven by microbes since birch seedlings
15 inoculated with nutrient poor sterilized red oak soil were smaller than control seedlings after one
16 growing season.

17

18 The increase in yellow birch diameter was noted despite similar rates of mycorrhizal infection
19 measured in the third growing season. In fact, adding forest soil did not influence mycorrhizal
20 infection of any AM or EM tree species. The number of seedlings excavated might have been too few
21 (5 per species per treatment) to detect an effect, especially since, in some cases, mycorrhizal fungal
22 colonization varied considerably within a treatment. Furthermore, tree seedlings produced in
23 nurseries can be naturally colonized by spore inoculation, but tree seedlings growing in forests may

1 have more and different mycorrhizal fungal species (Gagnon et al., 1991; Southworth et al., 2009).
2 As we conducted our study as a restoration project to test whether forest soil inoculation would affect
3 tree survival and growth, we did not identify the mycorrhizal fungal species. Also, we did not measure
4 the mycorrhizal fungal colonization within the first year. The differences in mycorrhizal infection
5 between soil treatments might be greater after one season of growth and then fade with time (Dickie
6 et al., 2007). This could explain the first year effects identified on EM tree species which benefited
7 from live forest soil inoculation compared to the addition of sterilized soil.

8
9 The greater responses of EM tree species than AM tree species are consistent with the literature. First,
10 the density of AM fungi increases with time since abandonment until a shift occurs in the plant
11 community from herbaceous vegetation to woody ectomycorrhizal hosts (Johnson et al., 1991). Thus,
12 AM fungi are suspected to be more abundant than EM fungi in old fields. Second, EM tree species
13 usually benefit more from mycorrhizal fungal associations than AM tree species, because exchange
14 between mycorrhizal fungi and trees are greater for EM species than AM species (van der Heijden
15 and Horton, 2009, Bradford, 2014) Third, soil nutrient availability in the field may have been more
16 beneficial to EM fungi than AM fungi. Mycorrhizal fungal colonization increases and provides more
17 benefits for seedlings in nutrient-limited environments, particularly in soils with low P availability in
18 the case of AM species and in soils with low N availability in the case of EM species (Jonsson et al.,
19 2001; Wiseman and Wells, 2005; Reynolds et al., 2005; Smith and Read, 2008). Although the low
20 organic C and total N levels of the agricultural soil was expected, we did not anticipate such a high
21 level of extractable P (i.e. 68 mg P kg⁻¹, whereas Sawyer et al. (2002) proposed that relative yields of
22 most crops are expected to be optimal between 26 and 35 mg P kg⁻¹, Mehlich III). As a whole, the
23 low molar N:P ratio of the agricultural soil suggests a low availability in N relative to P. This may
24 explain the few positive effects of forest soil inoculation, principally for AM tree species, as well as

1 the lack of positive correlations between mycorrhizal fungal infection and tree growth. Furthermore,
2 when a productive soil has low C levels, plant growth can be slightly reduced because the C demand
3 of mycorrhizal fungi and mycorrhizal associations can shift from a mutualistic to a parasitic
4 relationship (e.g. Johnson et al., 1997).

5
6 The study site was covered by AM grass and clover species. Since AM fungi are not host specific
7 (Klironomos, 2000; van der Heijden and Horton, 2009), AM tree seedlings may have been colonized
8 by AM fungi present in the agricultural field abandoned for more than 15 years. In addition, the
9 generalist red ash and red maple species are well adapted to these environments as they are among
10 the first to invade abandoned fields in our area (D'Orangeville et al., 2008). Open fields may contain
11 ectomycorrhizal propagules, but ectomycorrhizas (EM) are more abundant and diverse in forests
12 (Berman and Bledsoe, 1998). Our results suggest that restoration of EM tree species in abandoned
13 agricultural fields may benefit more from forest soil additions than AM tree species. Similarly, the
14 growth of an AM tree species (western red cedar seedlings) was improved by AM mycorrhizal
15 inoculum when planted in ectomycorrhizal-dominated hemlock-amabilis fir clearcuts (Guichon,
16 2015).

17

18 *4.2 The effects of soil provenance*

19 Contrary to our initial hypothesis, soil provenance somewhat influenced the results such as a positive
20 effect of red oak soil on yellow birch seedlings. Conversely, one EM tree species responded
21 negatively to the addition of forest soil collected under conspecific trees. Red oak seedlings inoculated
22 with red oak soil were smaller than those inoculated with yellow birch soil and control seedlings. This
23 negative feedback on red oak seedling height was also observed when sterilized red oak soil was
24 added, probably due to its low P content, 8 times lower than that of the agricultural field soil, as well

1 as lower exchangeable Ca, base saturation and pH (<5). The negative effect observed in the first year
2 following the inoculation of live red oak soil persisted through the second growing season. In addition
3 to its lower nutrient availability, it may be due to its pathogens because seedlings inoculated with
4 1500 ml of live red oak soil were smaller than those in the control, whereas those that had received
5 1500 ml of sterilized oak soil were no longer different from the control in the second year of growth.

6

7 This negative conspecific effect on red oak height was not as strong as what Packer and Clay (2000)
8 observed for the survival of *Prunus serotina* seedlings grown in pots filled with soils collected under
9 conspecific trees and affected by soil pathogens *Pythium* spp. Our result, observed on one out of 4
10 species, provides only weak support for the Janzen-Connell hypothesis (JCH). The JCH stipulates
11 that mature individuals of a same tree species increase the abundance in their vicinity of common
12 enemies potentially inducing a negative feedback on seedling survival and growth (Janzen 1970;
13 Connell 1971). The effect is considered to be mainly driven by soil microbes (Bever et al., 2010;
14 Mangan et al., 2010). Conversely, positive interactions due to ectomycorrhizal fungi were observed
15 on seedlings grown in soils collected under conspecific trees (Dickie et al., 2007; O'Brien et al.,
16 2011). In our experiment, the absence of significant effects for some tree species may be related to a
17 balance between the negative effects due to soil pathogens and the positive effects due to mutualist
18 species such as mycorrhizal fungi and N-fixing bacteria.

19

20 4.3 The effects of soil inoculum quantity, nutrient availability, and time

21 Generally, stronger (positive or negative) effects were observed with 1500 ml of forest soil inoculum
22 instead of 300 ml. The impact of soil inoculum quantity on tree growth could be explained by the
23 level of nutrient availability found in the forest soils compared to the agricultural field soil. For

1 example, the negative effects observed on EM tree growth when sterilized soil was added could be
2 due to the lower nutrient availability in EM forest soils, i.e. when more forest soil was added, seedling
3 roots likely had lower access to nutrients than the field soil due to a ‘‘dilution’’ effect. In contrast,
4 red maple seedlings inoculated with 1500 ml of live red ash forest soil, which has higher pH, Ca
5 levels and base saturation than the agricultural soil, were taller after one season of growth than those
6 that received only 300 ml.

7
8 A slight increase in red ash diameter following the addition of 1500 ml of live red maple soil or the
9 addition of sterilized red ash soil was also observed. In these cases, growth responses may have been
10 influenced by P levels in the added soils. Red maple soil (sterilized or not) has high levels of
11 extractable P (i.e. 40 mg kg⁻¹) and sterilized red ash soil (23 mg kg⁻¹, near optimal range) contains
12 more extractable P than the live red ash soil (8 mg kg⁻¹, within very low range) (see Sawyer et al.
13 (2002) for reference P levels (Mehlich III) for most crops). The difference in P levels between live
14 and sterilized red ash soil could be related to gamma irradiation which is highly effective for
15 sterilization, but may have some effects on soil chemical properties such as an increase in
16 exchangeable soil P levels (McNamara *et al.* 2003). The impact of gamma irradiation on forest soil
17 was higher for red ash soil probably due to the high activity of earthworms and microbes in this
18 Melanic Brunisol. On the one hand, following soil sterilization the dead earthworms and microbes
19 decomposed and thus likely increased soil P availability by creating a short-term pulse. On the other
20 hand, microbial recolonization of the soil will be required to promote mobilization of bound P
21 reserves.

22
23 The effect of soil inoculum quantity varied over time for EM tree growth. After one growing season,
24 negative effects on birch and oak seedlings were observed when 1500 ml of live soil was added, but

1 after three growing seasons, those seedlings had a larger diameter. Dickie et al. (2007) also observed
2 that oak seedling responses varied with the quantity of forest soil added as well as time. They showed
3 that first year seedlings receiving 2 litres of forest soil (instead of 200 ml or 0 ml) had a higher EM
4 infection rate but a lower shoot biomass, while third year seedlings had similar EM infection but a
5 higher leaf mass. The effect on initial aerial growth may be due to resource allocation to roots and
6 mycorrhizas.

7

8 Most effects observed after the first growing season did not persist. Generally, by the third year, tree
9 growth was similar in all treatments. In contrast to pot studies, inoculated microbes could have been
10 outcompeted by field microbes. The lack of persistent effects may also be due to tree roots extending
11 with time beyond the area where forest soil was added. Mechanical site preparation effects on EM
12 tree seedling growth have been found to disappear when roots grew beyond the modified patches in
13 undisturbed soils covered by AM herbaceous plants (Simard et al., 2003). In our study, third year
14 growth effects were only observed for yellow birch seedlings, the species with the smallest root
15 system (A. St-Denis, pers. obs.), previously inoculated with 1500 ml of live red oak soil.

16

17 **5. Conclusions**

18 Adding live forest soil to tree seedlings planted in abandoned agricultural fields did not affect tree
19 survival. Some positive effects of forest soil inoculation were observed for EM tree growth compared
20 to the addition of sterilized soil whereas few effects were observed on AM tree growth. The more
21 important effect was an increase in diameter after three growing seasons for yellow birch seedlings
22 inoculated with 1500 ml of red oak soil. Only one species responded negatively to the addition of
23 forest soil collected under conspecific trees. Future research should evaluate the reasons behind the

1 negative effect of conspecific soil on red oak growth by isolating and identifying soil pathogens. We
2 suggest that the lack of persistent effects of forest soil inoculation on tree seedlings was due to (1) the
3 possible presence of mycorrhizal fungal spores in agricultural soil and in the seedling potting-mix;
4 (2) the conflicting positive and negative effects of adding soil containing both possible beneficial and
5 antagonistic microorganisms; (3) the low organic C and high P levels of the agricultural soil; and (4)
6 the nutrient dilution effect of adding a soil inoculum with lower nutrients to the bulk soil with higher
7 nutrient availability. Because the effects of facilitation tend to increase as site fertility decreases,
8 notably in soils with low P availability in the case of AM species (Smith and Read 2008; Brooker et
9 al., 2008), a similar experiment may show more benefits if it was repeated on soils with lower P
10 availability (or lower nutrient availability in general) or on soils with low water retention capacity
11 (ex. sand or loamy sand soils with a very low clay fraction). Nevertheless, this study supports the idea
12 that planting EM tree species in abandoned agricultural field soils could benefit more from the
13 inoculation of forest soil microbes than AM tree species.

14

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24

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1 **Appendices**

2

3 **Table A.1.** ANOVAR results for the height and diameter of red ash, red maple, yellow birch and
4 red oak seedlings

5

6 **Table A.2.** ANOVA results for the height and diameter of red ash, red maple, yellow birch and red
7 oak seedlings (after the first season of growth)

8

9 **Fig. A.1.** Height and diameter (mean \pm sd) of red ash seedlings in the eight combined soil treatments
10 compared to the control.

11

12 **Fig. A.2.** Height and diameter (mean \pm sd) of red maple seedlings in the eight combined soil
13 treatments compared to the control.

14

15 **Fig. A.3.** Height and diameter (mean \pm sd) of yellow birch seedlings in the eight combined soil
16 treatments compared to the control.

17

18 **Fig. A.4** Height and diameter (mean \pm sd) of red oak seedlings in the eight combined soil treatments
19 compared to the control.

20