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Effects of Roadside Deposition on Growth and Pollutant Accumulation by Willow (*Salix miyabeana*)

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Abstract Roadside plants have the potential to accumulate pollutants and safeguard waterways. To assess growth and pollutant accumulation of roadside plants, the willow *Salix miyabeana* was grown (a) in a greenhouse on soil collected at different distances from an interstate highway to test the longer-term effects of pollutant deposition as manifested in soil, and (b) in the field on reference soil placed at different distances from that highway to test the shorter-term effects of proximity to pollutant sources during a single growing season. In the first experiment, relative growth rate (RGR) increased 150 % with distance of soil collection from the roadway, from a baseline near the highway to 100 m away. Relative nitrogen and phosphorus accumulation rates were positively correlated with RGR (P <0.0001), and total contents of zinc, strontium, copper, nickel, cadmium, and lead in new shoots were also positively correlated with RGR (P <0.05). Thus more rapidly growing plants accumulated more N, P, and metals. Reduced growth for plants grown on soils collected near the roadway was associated with very high tissue concentrations of sodium and soil concentrations of chloride, implicating the deposition of deicing agents in this northern temperate roadside ecosystem. In contrast, *S. miyabeana* showed the opposite pattern on reference soil in the field, with RGR decreasing 31 % as distance from the roadside increased. The latter trend appears to have resulted from greater soil moisture and reduced shading near the highway. We suggest that reducing road salt applications will promote growth and pollutant accumulation by roadside vegetation.

Keywords Roadside ecosystems · Highway runoff · Mitigation · Road salt · Metals · Plant mineral composition

1 Introduction

Roadside ecosystems are zones adjacent to roadways where traffic-related, non-point source pollutants may influence vegetation, wildlife, and biogeochemistry (Hamilton and Harrison 1991, OECD 1994; Forman et al. 2003). This influence may extend tens or hundreds of meters from roadways (Hofstra and Hall 1971; Legret and Pagotto 2006; Bettez et al. 2013; Redling et al. 2013). Coupled with our vast networks of roadways—comprising over 4 million km of pavement in the U.S. (USDOT 2011)—these narrow ecosystems total a substantial area. If delimited as just 20 m wide, roadside ecosystems in the U.S. approximate the area of the state of South Carolina.

The ability of these systems to process traffic-related pollutants can contribute to safeguarding the quality of our freshwater resources (Yu et al. 2001; Stagge et al. 2012), which will take on increased importance as traffic volume and pressure on those resources grow. Traffic-related pollutants include plant nutrients, potentially toxic

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metals, and deicing salts. Nitrogen (N) and phosphorus (P), both key mineral nutrients in the eutrophication of surface waters, may be elevated in roadside ecosystems—N because vehicles emit both ammonia and N oxides (e.g., Bettez et al. 2013) and P because of elevated levels in surface runoff (Wu et al. 1998). Metals can be derived from fuels, lubricants, vehicle wear, and pavement erosion (Davis et al. 2001; Forman et al. 2003). Salts are long-established pollutants where road deicing is common in winter or salts are applied for dust control (Lacasse and Rich 1964; Kaushal et al. 2005).

Roadside vegetation falls under the influence of atmospheric deposition of vehicle emissions and salt spray, as well as surface runoff from roadways. The impact of these pollutants, whether positive (nutrients) or negative (metals, salts), is likely to be greatest near roadways and to decline with increasing distance (Lacasse and Rich 1964; Viskari and Kärenlampi 2000; Bettez et al. 2013). A potentially important biogeochemical role of roadside vegetation is to accumulate pollutants, at least during the growing season, and thus mitigate surface water and/or groundwater pollution (e.g., Stagge et al. 2012).

Our overall goal for this study was to assess the growth and pollutant accumulation of roadside plants as they are affected by prevailing environmental conditions—including the pollutants themselves. To this end, we chose a focal plant species, essentially as a bioassay organism. A more specific goal was to distinguish between the effects of longer-term pollutant accumulation, as manifested in the soil located at different distances from a roadway, and the shorter-term location-specific effects of atmospheric deposition and surface runoff examined during one growing season.

These goals led to two specific questions. First, how does soil collected at different distances from an interstate highway influence the growth and pollutant accumulation of plants under controlled conditions in a greenhouse? Second, how do conditions prevailing during the growing season at different distances from that highway affect the growth and pollutant accumulation of plants grown in reference soil collected far from the highway?

An additional objective afforded by these experiments was to evaluate relationships between plant growth and (a) elemental composition and (b) measured environmental conditions.

2 Materials and Methods

2.1 Site and Species Description

Interstate 81, which extends northward from eastern Tennessee to the Canadian border, passes through Binghamton, NY, just a few kilometers north of the Pennsylvania border. This section, completed in the mid-1960's, supports an average annual daily traffic flow of ca. 70,000 vehicles (NYSDOT 2010) within a metropolitan area of ca. 200,000. Our study site, located at 42°7.2′ N and 75°54.5′ W, lies immediately to the east and southeast of the northbound lane of I-81, approximately 500 m north of NY 17. It is a main thoroughfare running north–south through the city. This region of NY experiences a temperate climate, receiving approximately 100 cm of rainfall annually; 26.7 cm of rain fell during the experimental duration from early June to mid-August, 2009 (US Climate Data 2014).

We established a baseline parallel to and ca. 10 m from the roadway, and six transects running perpendicularly to this baseline. These six transects, each 100 m long, were parallel to each other at regular 15 m intervals. Soil for plant growth experiments was collected either at different distances from the baseline along each of the transects or from a reference location in a second growth oak forest within the Binghamton University Nature Preserve (42°5′ N, 75°59′ W)—ca. 300 m from a local road with relatively light traffic flow. Soil collected from the I-81 site had a mean organic matter content of 6.0 %, and mean weighted soil pH ranged from 5.6 at the 100 m distance to 7.5 close to the highway. Soil collected from the Binghamton University Nature Preserve had a mean organic matter content of 16.0 % and a mean weighted soil pH of 4.1.

Our experimental organism was the willow *Salix miyabeana* Seemen (Salicaceae), a deciduous shrub or small tree. The cultivar SX 64 is a hardy, fast-growing plant which has been tested for stormwater treatment (Mirck and Volk 2010) and biomass production (Quaye and Volk 2013). We used dormant stem cuttings ca. 25 cm long, which were randomly assigned to the two experiments described below and for determination of initial fresh mass: dry mass ratio.

2.2 Experimental Design and Planting

To evaluate the longer-term effects of traffic-related pollutants on the growth and pollutant accumulation of

Salix miyabeana, we collected soil at four distances from the baseline near the roadway—0, 10, 30, and 100 m—along each of the six transects. The 24 soil samples, each ca. 15 L, were transported to the Binghamton University Research Greenhouse and placed in pots. After determination of its initial fresh mass, a randomly selected willow stem was planted in each pot on 4 June 2009. Each of the 24 pots was watered weekly with 1 L of deionized water and regularly rotated in position on the greenhouse bench to minimize pot-to-pot variations in light and temperature regimes. Henceforth, we refer to this as the "soil experiment" because it focuses on soil as the key variable.

To evaluate the shorter-term effects of field location on willow growth and pollutant accumulation *separately* from soil effects, we collected six batches of reference soil from the oak forest and mixed each batch thoroughly before allotting it to four 15 L pots. The four pots from each batch were assigned to one of the perpendicular transects at the Interstate 81 field site, and placed in the holes existing from the previous soil collection at 0, 10, 30, and 100 m from the baseline. Thus each transect with its four pots comprised a block, with a different soil batch for each block. Again, after determination of its initial fresh mass, a randomly selected willow stem was planted in each pot on 8 June 2009. The 24 pots were watered in situ initially to aid in transplanting success, and thenceforth were subjected to natural location-specific variations in water availability. We refer to this as the "location experiment".

The stems which were not assigned to either of the two experiments were weighed, then dried to constant mass at 60 °C. Determination of the fresh mass: dry mass ratio for these stems allowed estimation of initial dry mass for all experimental stems, and ground stems were analyzed to determine initial N and P content.

As the location experiment progressed, it became evident that soil was consistently more moist near the roadway—apparently due to runoff from the highway—and neighboring plants tended to cast more shade on our experimental plants at greater distances from the highway. To quantify these trends, we collected soil samples and assessed shading semi-quantitatively in mid-August for each of the 24 locations. Soil samples were returned to the lab, weighed, then dried to constant mass at 105 °C for determination of water content (expressed as a % of dry soil mass). At each location, categories of shading were visually estimated based on the herbaceous vegetation (a) within a 1 m^2 square quadrat centered on each experimental pot, and (b) outside this quadrat, but potentially casting shade on experimental plants, particularly from the southeast, south, and southwest. For each, we rated shading by visual observation on a scale of 0 (no shading) to 3 (substantial shading) and added the two measures together to yield our shade index. None of the plants were heavily shaded from above even at the end of the experiment.

2.3 Harvesting and Analysis

2.3.1 Plant Harvest and Tissue Analysis

Plants were harvested after 71 days for the soil experiment and 77 days for the location experiment, separated into original stems, coarse roots, and new leafy shoots, and soil mass was determined. Fine root biomass was estimated by thoroughly mixing the soil for each pot and then, for each pot, sieving a 100-g soil subsample through a 4-mm soil sieve, cleaning and drying the fine roots in each subsample, and extrapolating to the total soil mass for each pot, which averaged ca. 11 kg. Dry mass for all plant fractions was determined after drying in paper bags to constant mass at 60 °C. Stems and new shoots were ground to fine powder with a stainless steel Wiley Mill with a #40 mesh screen (0.43 mm openings) and stored in glass scintillation vials until tissue analysis. Soil corresponding to each of the 48 willows was retained in cold storage (5 °C) until subsequently analyzed.

Relative growth rate (RGR) was calculated according to formula (1):

$$\mathrm{RGR} = (\ln B_{\mathrm{f}} - \ln B_{\mathrm{i}})/t \tag{1}$$

Where B_{f}=final plant dry mass (g), B_{i}=initial plant dry biomass (g), and t=length of experiment (days).

To determine the N and P content of ground plant tissues, 200 mg samples were digested in a solution matrix of concentrated H_2SO_4 and 30 % H_2O_2 for 2.5 h in a Tecator system 4.0, 1016 block digester. Selenium and $LiSO_4$ were added to enhance the digestion efficiency (Allen 1989). Cooled digests were diluted with Nanopure water to 2 % H_2SO_4 before being run on a Lachat QuickChem flow-injection autoanalyzer 8000 series to determine total Kjeldahl N (TKN, Method 10-107-04-3-P) and total P (TP, Method 10-115-01-1-A) concentrations.

Relative nitrogen accumulation rate (RNAR) and relative phosphorus accumulation rate (RPAR) were calculated in the same way as RGR, except that they were based on total N and P content of stems plus new shoots at the end of the experiment in relation to initial stem N content. Formula (2) details the calculation for RNAR:

$$\mathrm{RNAR} = \left(ln\, N_f - ln\, N_i\right)/t \quad (2)$$

Where N_f=final aboveground plant N content (mg), N_i=initial stem N content (mg), and t=length of experiment (days).

An analogous formula was used to determine RPAR.

We selected the following metals for focus, because they are all potentially toxic and are linked to roadway pollution (e.g., Granato 1996; Forman et al. 2003): vanadium (V), nickel (Ni), copper (Cu), zinc (Zn), strontium (Sr), cadmium (Cd), and lead (Pb). We also focus on sodium (Na) as a prime constituent of road salt in the Binghamton area. For the purposes of this paper, we refer to V, Ni, Cu, Zn, Sr, Cd, and Pb (with atomic mass ranging from 50.9 to 112.4) as the "heavier metals" to distinguish them from Na (atomic mass 23).

To determine metal content, ground plant tissues were microwave digested using an ETHOS E microwave digester made by Milestone Microwave Lab Systems, and10 % trace metal grade nitric acid (US-EPA Method 3051a). Digested tissues were diluted with Nanopure water and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) on a Perkin Elmer Elan 6000 following US-EPA Method 200.8. Metal concentrations were expressed as milligrams of metal per kilogram dry plant tissue. Total metal content (milligrams of metal in new shoots) was determined for each plant as the product of tissue metal concentration and new shoot dry mass.

2.3.2 Soil Analysis

Each of the 48 soil samples was analyzed for moisture content (as above, water mass as a % of dry soil mass after drying at 105 °C) and extractable Cl. Soil Cl was extracted from 20 g fresh soil samples using 50 mL of Nanopure water. Samples were shaken using a reciprocating shaker for an hour, then allowed to settle for at least 4 h in cold storage (5 °C). Settled samples were gravity filtered through ashed Whatman GF/A glass microfiber filters. Filtrate was acidified with 50 % H_2SO_4 to yield a 2 % H_2SO_4 solution and placed in cold storage until analysis. Chloride concentrations were determined using a Lachat QuickChem flow-injection autoanalyzer 8000 series (Method 10-117-07-1-A) and then expressed as mg Cl per kilogram dry soil.

2.3.3 Statistical Analysis

One-way analysis of variance (ANOVA, alpha=0.05) was performed using IBM SPSS version 19 (SPSS Inc., Chicago, IL, USA) on RGR, RNAR, RPAR, tissue Na concentration and soil Cl concentration datasets—each with four distance-related treatments replicated six times. Tissue Na concentration and soil Cl concentration datasets were log transformed due to violations of the equal variance assumption. Post hoc comparisons with Tukey's Honest Significant Difference (Tukey HSD) test were used to test for differences among distance treatments. Linear regressions were performed using Microsoft Office EXCEL between RGR and RNAR, RPAR, tissue metal concentration, and tissue total metal content—all with a sample size of 24.

3 Results

3.1 Soil Experiment

Relative growth rate increased monotonically with distance from the roadway for *Salix* grown in the greenhouse on soil collected from the I-81 site (Fig. 1), by 150 % from 0 m (RGR=0.0048 day^{-1}) to 100 m (0.0118 day^{-1}, $F_{3,20}$=3.358, P=0.039). Tissue N concentration averaged 20.6 mg/kg, decreasing monotonically (and insignificantly according to one-way ANOVA) from 22.0 to 19.5 with increasing soil collection distance from the roadway. Tissue phosphorus (P) concentration averaged 1.13 mg/kg, also decreasing (insignificantly) with increasing distance from 1.70 to 0.74 mg/kg. On an individual plant level, RNAR and RPAR were both highly significantly correlated to RGR (Fig. 2). Thus, more rapidly growing plants also accumulated N and P more rapidly. These accumulation rates followed a similar distance pattern to RGR, tending to increase with increasing distance from the roadway (Fig. 2).

Mean metal concentrations in new shoots ranged from 0.15 mg/kg (V) to 147 mg/kg (Zn) for the heavier metals, with a far higher value (3054 mg/kg) for Na

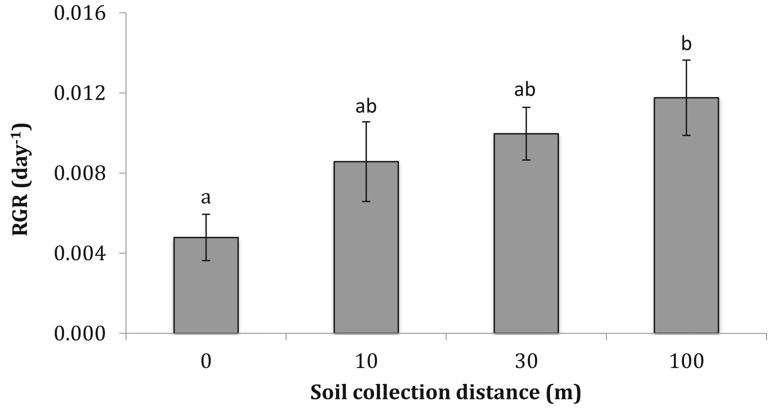


Fig. 1 Relative growth rate (RGR) for plants grown in the greenhouse on soil collected at different distances from the highway. Means, shown with standard errors (n=6), differ significantly if they do not share a common letter (based on post-hoc Tukey means comparison test, P <0.05)

(Table 1). On a treatment basis, highest mean metal concentrations in new shoots for the seven heavier metals were observed in *Salix* planted in soil collected closest to the highway (0 m, data not shown). However, this difference was only significant for Cu (one-way ANOVA, P <0.01). On an individual plant basis, none of these levels were significantly correlated with RGR (Table 1).

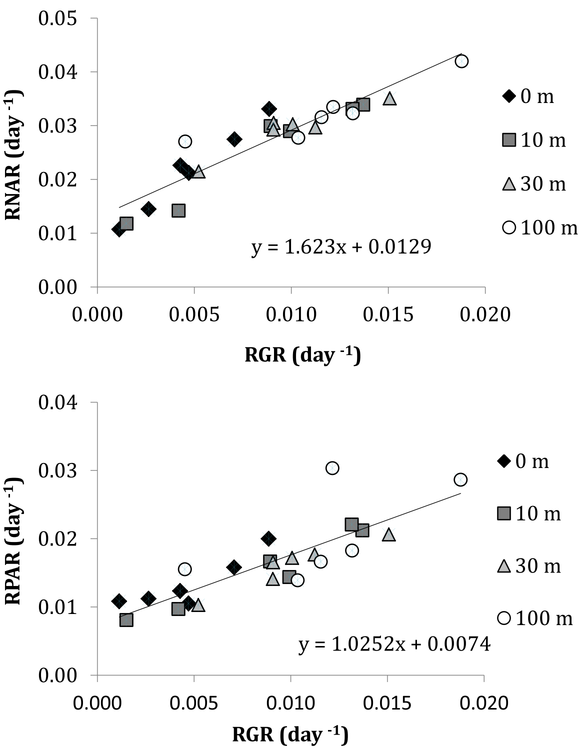


Fig. 2 Relative nitrogen accumulation rate (RNAR r=0.93, P <0.0001, n=24) and relative phosphorus accumulation rate (RPAR r=0.84, P <0.0001, n=24) vs. relative growth rate (RGR) for willows grown in soil collected at different distances (0, 10, 30, and 100 m) from the highway

Mean total metal content was by far the highest for Na (Table 1, 6.69 mg/plant). For the heavier metals, total tissue content in the new shoots ranged from relatively high values for Zn and Sr (1.01 and 0.65 mg/plant, respectively) to 0.7 μg/plant for V. Total metal content in the new shoots ranged over at least an order of magnitude for each of the heavier metals and nearly 700-fold for Na (Table 1). On an individual plant basis, total metal content in the new shoots was positively correlated with RGR for all seven of the heavier metals, significantly so in five cases (Table 1). In contrast, there was a highly significant negative correlation between RGR and Na content (Table 1). Thus, with the exception of Na, plants gaining biomass more rapidly also accumulated more metals in their young leafy shoots.

Tissue concentrations of Na in the new shoots showed a clear pattern when expressed as a function of distance from the highway, being significantly higher for plants grown on soil collected closer to the highway (6229 mg/kg plant at 10 m) than for those grown on soil collected at 100 m (58 mg/kg, Fig. 3; $F_{3.20}$=5.252, P= 0.008). Extractable soil Cl showed essentially the same pattern, declining from 33.9 mg/kg in 10 m soil to <0.01 mg/kg in 100 m soil (Fig. 3; $F_{3.20}$=7.544, P= 0.001).

3.2 Location Experiment

In the field on reference soil, RGR showed the opposite pattern to that recorded in the soil experiment: RGR *decreased* monotonically 31 % from 0.0208 day^{-1} at 0 m to 0.0143 day^{-1} at 100 m (Fig. 4; $F_{3.20}$=3.273, P= 0.042). At the I-81 site, greater soil moisture ($F_{3.20}$= 2.601, P=0.080) and less shading ($F_{3.20}$=16.39, P<0.001) were observed closer to the highway

Table 1 *Salix* tissue concentrations and total content for metals in new shoots of plants grown in the greenhouse on soil collected at the highway site. Means shown with standard errors (n=24) for concentration and total content; range is given for total content. Pearson correlation coefficients (r) and P values are from linear regressions between RGR and metal concentration and between RGR and total metal content

	Concentration			Total content			
	Mean	RGR vs. mg/kg		Mean	Range	RGR vs. mg/plant	
Metal	(mg/kg)	r	P	(mg/plant)	(mg/plant)	r	P
Zn	147.1±11.4	0.06	0.780	1.01±0.14	0.04–2.32	0.61	0.002
Sr	97.7±11.8	−0.07	0.743	0.652±0.110	0.068–1.784	0.40	0.055
Cu	9.40±0.62	−0.31	0.136	0.059±0.008	0.011–0.144	0.56	0.004
Ni	3.04±0.46	−0.37	0.077	0.017±0.002	0.003–0.045	0.50	0.012
Cd	1.79±0.14	0.09	0.677	0.012±0.002	<0.001–0.028	0.60	0.002
Pb	1.30±0.22	−0.28	0.180	0.0076±0.0012	<0.0001–0.0232	0.47	0.020
V	0.15±0.05	−0.33	0.120	0.0007±0.0001	<0.0001–0.0027	0.13	0.534
Na	3054±1133	−0.19	0.385	6.69±1.95	0.04–28.63	−0.60	0.002

(Fig. 5). Tissue N concentration averaged 14.2 mg/kg, and tissue P concentration averaged 0.72 mg/kg. As in the soil experiment, there were no significant effects of distance on these tissue concentrations according to one-way ANOVA. Also like the soil experiment, RNAR and RPAR were both highly significant and positively correlated to RGR (RNAR=0.845 RGR+0.015, r=0.71, P <0.0001; RPAR=0.989 RGR+0.003, r=0.83, P <0.0001). More rapidly growing *Salix* accumulated N and P more rapidly. Again, accumulation rates followed a similar distance pattern to RGR, in this case with greater values for plants grown closer to the highway.

Mean tissue concentration and total tissue content for the seven heavier metals followed the same order as in the soil experiment (Table 2): Zn had the greatest values and V the lowest. Total tissue Na averaged 0.38 mg/plant, far lower than the 6.69 mg/plant observed in the soil experiment. Total tissue metal content in the new shoots was positively, but not significantly, correlated with RGR for seven of the eight metals (Table 2), suggesting a tendency for faster growing plants to accumulate more metals in their new shoots.

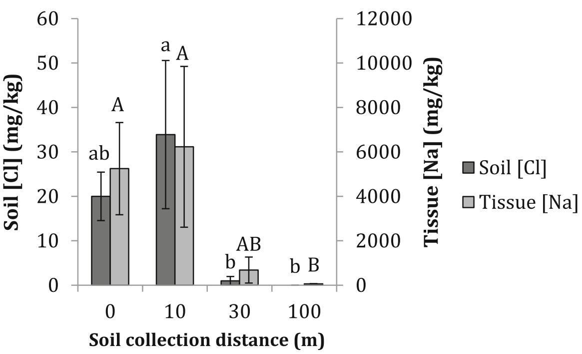


Fig. 3 Extractable soil Cl and Na concentration in new shoots for plants grown on soil collected at different distances from the highway. Means, shown with standard error bars (n=6), differ significantly if they do not share a common letter (based on post-hoc Tukey means comparison test, P <0.05)

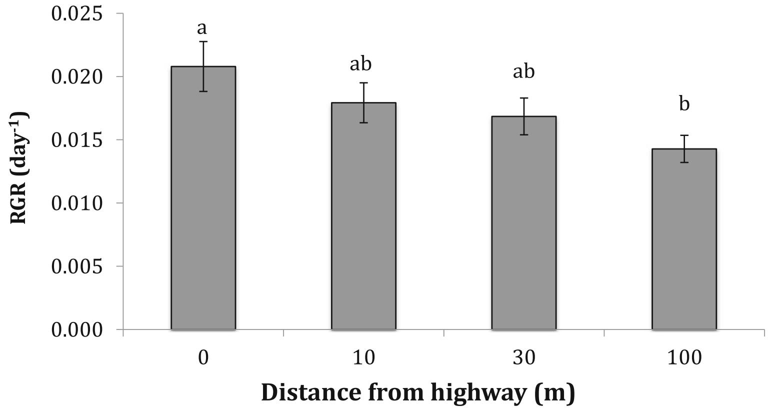
4 Discussion

4.1 Growth Drives Pollutant Accumulation

Accumulation of pollutants by *Salix miyabeana* was growth-dependent for the macronutrients N and P and for the heavier metals focused on in this study (Zn, Sr, Cu, Ni, Cd, Pb, and V). In both soil and location experiments, plants with greater growth rates also exhibited higher rates of N and P accumulation, as evidenced by the highly significant correlations between RGR and both RNAR and RPAR (e.g., Fig. 2).

Greater quantities of the heavier metals were also accumulated by the more rapidly growing plants in the soil experiment, as evidenced by the positive correlations between RGR and total metal content in new shoots of *S. miyabeana* (Table 1). These correlations were significant (P=0.002–0.020) for Zn, Cu, Ni, Cd, and Pb. The same correlations were positive for six of the seven heavier metals in the location experiment (Table 2), but none were significant at the 0.05 level.

Fig. 4 Relative growth rate (RGR) for plants grown on reference soil at different distances from the highway. Means, shown with standard errors (n=6), differ significantly if they do not share a common letter (based on post-hoc Tukey means comparison test, P <0.05)



Both Ni and V approached significance, with P<0.10. The greater significance for these correlations observed in the soil experiment may stem in part from its greater range of RGR, variation in which drives variation in metal accumulation. RGR varied 2.5-fold in the soil experiment, but only 1.5-fold in the location experiment. In any case, in both experiments, *Salix* were able to accumulate roadside metals as the plants increased in biomass. This finding supports the results of previous studies that found *Salix* to be a promising phytoremediation tool in contaminated ecosystems (Landberg and Greger 1996; Pulford and Watson 2003).

For the macronutrients N and P in both experiments and the heavier metals in the soil experiment, it seems likely that greater pollutant accumulation reflects more extensive root systems. Faster growing plants had greater root biomass (data not shown), which presumably had intimate contact with a greater volume of soil.

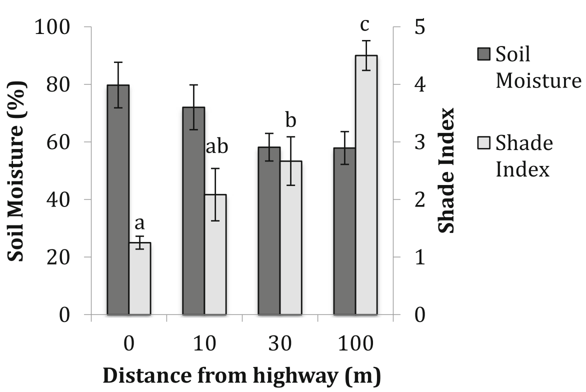


Fig. 5 Soil moisture and shade index values for plants grown in the field at different distances from the highway. Means, shown with standard errors (n=6), differ significantly if they do not share a common letter (based on post-hoc Tukey means comparison test, P <0.05)

Accumulation of Na did not parallel that of the other metals in the soil experiment. The maximum observed Na accumulation in new shoots (28.63 mg) was over an order of magnitude greater than that for Zn (2.32 mg), the most concentrated of the heavier metals (Table 1). More striking was the *negative* correlation between RGR and Na content—just the opposite pattern to that shown for N, P, and all seven heavier metals. This correlation is a consequence of reduced RGR for plants grown on soil collected from near the highway (Fig. 1) coupled with their far greater tissue concentrations of Na (Fig. 3). In contrast, Na accumulation by plants in the location experiment averaged only about 5 % of that in the soil experiment (Tables 1 and 2), and RGR was positively (but not significantly) correlated with Na accumulation in new shoots.

4.2 Growth Rate and Proximity to the Highway

As the above data show, plant growth rates are central to pollutant accumulation, and indeed may drive that accumulation. Our two experiments yielded contrasting patterns for the effect of proximity to the highway on growth rates, and thus on pollutant accumulation. In relation to their very similar RGR's for the 100-m treatments most distant from the highway (0.12 day^{-1} for the soil experiment, Fig. 1; 0.14 day^{-1} for the location experiment, Fig. 4), willows in the location experiment showed growth *enhancement* near the highway, whereas willows in the soil experiment showed growth *reduction* on soil collected near the highway. Below, we present correlates of these opposing trends.

In our location experiment, we found no detrimental influence of traffic-related pollutants, whether from surface runoff or atmospheric deposition, on willow growth

Table 2 *Salix* tissue concentrations and total content for metals in new shoots of plants grown in reference soil at the highway site. Means shown with standard errors (n=24) for concentration and total content; range is given for total content. Pearson correlation coefficients (r) and P values are from linear regressions between RGR and total metal content

	Concentration	Total content			
	Mean	Mean	Range	RGR vs. mg/plant	
Metal	(mg/kg)	(mg/plant)	(mg/plant)	r	P
Zn	638.9±54.1	8.84±1.09	2.55–21.81	0.07	0.571
Sr	61.28±4.87	0.834±0.089	0.345–1.803	0.04	0.836
Cu	7.37±0.67	0.099±0.009	0.032–0.201	0.12	0.571
Ni	5.31±0.361	0.075±0.008	0.019–0.159	0.36	0.083
Cd	4.29±0.47	0.058±0.008	0.008–0.147	−0.14	0.506
Pb	0.692±0.095	0.0094±0.0013	0.0023–0.0241	0.27	0.194
V	0.028±0.011	0.0004±0.0001	<0.0001–0.0021	0.37	0.078
Na	26.87±4.81	0.38±0.09	<0.01–2.28	0.37	0.077

during a single growing season. On the contrary, plants grown in close proximity to the highway (on reference soil) actually grew faster than those planted farther away (Fig. 4). We attribute this pattern to greater soil moisture near the highway, which we observed repeatedly during this field experiment and measured at its conclusion, and to less shading from neighboring plants (Fig. 5). The reduced shading near the highway probably reflects limitations of roadside soil on the growth and stature of the plants previously established in the roadside ecosystem.

Managing for greater growth of roadside vegetation so as to promote pollutant accumulation hinges on understanding what limits growth on near-roadside soil—willows grown on soil closest to the highway realized only 40 % of the growth rates of those grown on more distant soil (Fig. 1). We assume this soil effect reflects the accumulation of traffic-related pollutants over the years. In an attempt to account for this observed growth limitation in the soil experiment, we address macronutrients, the heavier metals, and deicing salts in turn.

The macronutrients N and P, with mean tissue concentrations in plants grown on soil collected closest to the highway of 22.9 and 1.70 mg/kg, respectively, were at levels considered adequate for N but potentially limiting for P (Epstein and Bloom 2005)—"potentially" because we do not have specific information on limiting levels for *S. miyabeana*. It is possible that P limitation contributed to the lower RGR for these plants, but we consider this unlikely because tissue P concentrations were lowest in the faster growing plants grown on more distant soil in this experiment.

Concentrations of metals in plant tissues can provide a clue as to whether or not metal toxicity limits plant growth. Plant tissue concentrations of Zn were the highest of the heavier metals we observed in our soil experiment (Table 1). This matches other studies examining metal tissue concentrations in *Salix* (Landberg and Greger 1996; Nissen and Lepp 1997; Stoltz and Greger 2002) and may follow from greater deposition rates of Zn as compared to metals such as Cu, Pb, and Cd (e.g., Legret and Pagotto 2006).

Our Zn, Cu, Ni, Cd, and Pb tissue concentrations for *Salix* grown in roadside soil were within and in some cases above the ranges reported for *Salix* and other plants grown on a variety of contaminated substrates, including industrially polluted soil contaminated with heavy metals (Landberg and Greger 1996), mine tailings (Stoltz and Greger 2002), sewage sludge (Sauerbeck 1991; Pulford et al. 2002), and landfill (Green et al. 2014).

Bioavailability of metals in soil may account for some of the differences in tissue metal concentrations between our two experiments. In roadside ecosystems, bioavailability of some metals may be limited by the higher soil pH in sites closer to roadways (e.g., Jim 1998; Green et al. 2008), a pattern we observed at our highway site. Sauerbeck (1991) reported that Zn, Ni, and Cd concentrations in plants grown on metal-contaminated soils—unlike Cu and Pb—were higher in plants grown on more acidic soils. Indeed, those are the same three metals occurring in higher tissue concentrations in our location experiment (Table 2) on more acidic, reference soil (pH 4.1) than on the more neutral roadside soil (pH 5.6–7.5) from our soil experiment (Table 1). Particularly for Zn, the tissue concentration differences lead to much more total metal accumulation for plants grown on the reference soil.

Metal pollutants can cause plant mortality or decreased growth due to alterations of plant metabolism (Nagajyoti et al. 2010). We do not, however, have any clear evidence that these metals limited *Salix* growth in our soil experiment because RGR did not show any significant negative correlations with tissue metal concentrations (Table 1). Indeed, the correlation was slightly positive for Zn and Cd.

In the soil experiment, higher Na and Cl concentrations were observed in the *Salix* tissues and the soil, respectively, closer to the roadside (Fig. 3). These high tissue concentrations of Na were more than two orders of magnitude greater than we observed in the location experiment. We assume the latter were so much lower because of the use of reference soil not subject to saline highway runoff.

The adverse effects of salts on plants closer to highways have long been known (e.g., Lacasse and Rich 1964). In our study, tissue Na levels in new shoots of *Salix* grown on soil collected from near the highway are similar to values that have been associated with leaf injury for other tree species (Hofstra and Hall 1971; Hall et al. 1973; Viskari and Kärenlampi 2000), although we did not note any visible symptoms in these plants.

5 Conclusion

The potential for roadside plants—in this case a willow species used as an assay organism (*S. miyabeana*)—to accumulate traffic-related pollutants depends on their ability to grow rapidly. Longer-term impacts of pollutant deposition from an interstate highway, as manifested in the soil, substantially reduced growth rate, and thus accumulation of the macronutrients N and P, as well as the metals V, Ni, Cu, Zn, Sr, Cd, and Pb. These soil effects can be attributed, at least in part, to runoff of deicing salts but not to the heavier metals of interest.

In our field location experiment, we observed a stimulation of plant growth in proximity to the highway for plants grown for a single season on reference soils collected far from the highway. These plants appeared to respond positively to greater soil moisture and reduced shading near the roadway, thus reinforcing the stance that roadside soils limit growth and pollutant accumulation.

If roadside salt levels can be minimized, *Salix* grown near roadways can increase in biomass and accumulate deposited nutrients and metals and thus potentially safeguard waterways from such pollutants.

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