The Effect of *Fallopia japonica* on Soil Bacterial Diversity

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Introduction

Fallopia japonica, or Japanese knotweed, carries a bamboo-like appearance, but don't let this fool you. The plant is not necessarily poisonous, but extremely invasive. *Fallopia japonica* was first discovered in the North America, in the late 19th century (Rhoads & Block, 2002). Now the plant can be found in Canada provinces and Alaska. The knotweed also has a long history with invasion impacts in Europe, and is considered to be the most invasive in the United Kingdom (Remaley, 2009). *Fallopia japonica* spreads very quickly, and forms dense thickets that remove native species.

Though it doesn't affect germination rates (Remaley, 2009), Japanese Knotweed affects the rate at which other plants grow. Certain plants (and possibly *Fallopia japonica*) use allelopathy, which is an action by which organisms produces one or more biochemicals that influence the growth, survival and reproduction of other organisms. The plant roots secrete carbon and chemical ions into soil and create spaces that are suited for specific microbes that would help the growth of the plant itself (Burke & Chan, 2009). Previous studies including one by Burke and Chan (2009) focused on the effect of plant roots on soil microbes. They tested a plant called *Alliaria petiolata*, or Garlic Mustard. The results showed that there was a significant temporal change in forest soil bacterial communities with the litter of the plant.

I chose to work on this question because Japanese Knotweed has been a problem that my Grandma has had for a long time. I would usually have to work to get rid of the plant, but it would keep coming back. What was interesting is that little or nothing significantly grows near it. This evolutionary advantage, though good for the plant, is not good for anything else. To be able to control the plant would be good for every living organism, including my Grandma! To start the research on how to control the plant, we must make small steps to understand how the plant works. All research we make can be integrated to fo

http://www.eattheweeds.com/wp-content/uploads/2011/11/japanese-knotweed.jpg

Methods

Paired soil samples were collected from 3 locations between 5-20 cm depth in the rooting zone. At each site 3 samples were collected from knotweed areas and 3 from areas without knotweed. Restriction Fragment Length Polymorphism (T-RFLP) was used to compare bacterial communities at these two distinct plant communities (Liu, et al., 1997).

I extracted DNA using the MoBio PowerSoil DNA Isolation kit following kit protocols (MoBio, 2010). Bacterial DNA was then amplified in a 25 uL reaction containing GE healthcare PuReTaq Ready-To-Go PCR beads. 0.5 uM concentrations of each forward and reverse primer (5'-/5HEX/CCT ACG GGA GGC AGC AG-3' and 5'-TAC CTT GTT ACG ACT T-3', respectively). 5 ul of DNA template was used per PCR reaction, with a negative control run to detect artficial PCR product. Thermocycling conditions (Biorad) were as follows: initial denaturation for 5 min at 95ºC followed by 34 cycles at 94ºC for 1 minute, 50ºC for 1 minute, and 72ºC for 2 minutes and 30 seconds. PCR product was stained in SYBRGreen and viewed using the Vernier TransIlluminator (Figure 1).

Restriction digestion of PCR-products occurred at 37ºC for 3 hours using the enzyme MspI. Restriction fragments were electrophoresed on a 1% agarose gel and stained with SYBRgreen. Capillary electrophoresis was completed by the UAF CORE Lab. Peak analysis was conducted using Peak Scanner software, with each individual peak presumed to equate a single species. Small peaks (<100) were discounted as background noise.

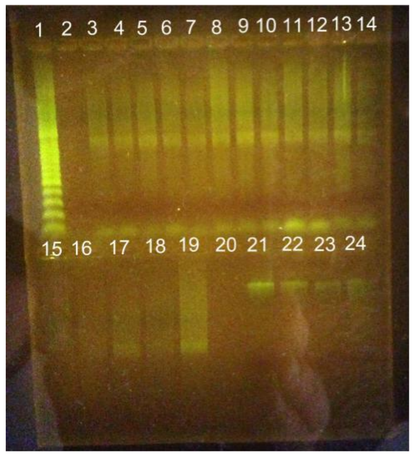

Figure 1: Agarose gel of PCR product in lanes 2-19; genomic DNA in lanes 21-24

Results

Site 1, (no knotweed)had a lower mean number of bacteria (33.5, range 25-42) than the knotweed community (mean 59, range 54-64) (Figure 2). Sites 2 and 3 had a greater number of bacteria in the no knotweed communities (means of 60, range 40-73, and 94, range 94-94, respectively compared to 45, range 31-56, and 33, 29-37 range). A student t-test of difference between the means in sites 2 and 3 produced a p-value of 0.076. The comparison of means at knotweed verses no knotweed at all sites was not significant (p=0.36).

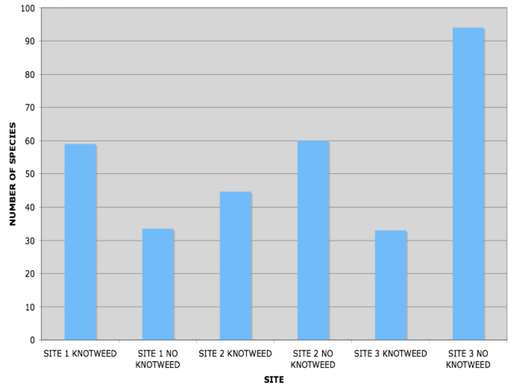

Figure 2: Comparison of soil bacteria diversity in paired knotweed - no knotweed sites.

When electropherograms were compared manually on the Peak Scanner software, there was little difference in the types of species observed between sites with and without knotweed. Not all pairwise comparisons were made.

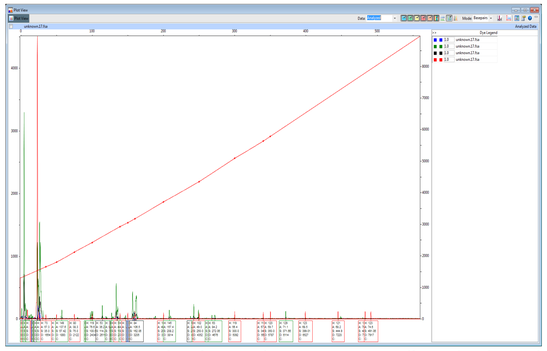

Figure 3: Sample electropherogram. Small peaks (<100) were discounted as background noise; each peak was presumed an individual species.

Conclusions

I hypothesized that due to Japanese Knotweed's allelopathic properties, the bacterial communities would change. This was rejected, since there was no significant change in the diversity of the bacteria of the soil near the Japanese Knotweed when compared to soil away from the knotweed (p=0.36). Interestingly, the number of species of bacteria (in site 1) was less in the soil with no knotweed in close proximity when compared to the soil near the knotweed; in sites 2 and 3, the opposite occurred. Overall, the mean number of species was larger where no knotweed was present, particularly in site 3.

A possible source of error is the time of collection. The knotweed was wilting at the time of soil collection, and the knotweed is more active during the spring and summer. Another source of error is how heterogeneous soil is: accuracy comes with the quantity of the soil. To truly get at the differences in soil bacterial communities, a larger sample size would be necessary. Moreover, a thorough analysis using software would mine the data for more information.

The purpose of this project is to contribute to the research on how Japanese knotweed influences the ecosystem, since the plant is extremely invasive. Another study of invasive plants suggests that certain plants do change microbial communities (Burke and Chan, 2009). Further research on this question would be helpful in learning more about why we have the results we obtained.

Literature Cited

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