

SOIL IMPACTS DUE TO THE INVASION OF ILEX AQUIFOLIUM (ENGLISH HOLLY) INTO SECOND GROWTH FORESTS OF THE PACIFIC NORTHWEST

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Abstract

Pacific Northwest temperate rainforests are generally distinctive in their resistance to invasive species. Nevertheless, a novel evergreen tree species, *Ilex aquifolium*, also known as English Holly, is increasingly recognized as a successful invasive tree in undisturbed Pacific Northwest forests. One of the characteristics that makes this invasive plant so unique is its ability to readily establish itself in otherwise undisturbed forests. Nevertheless, no studies have examined the ecosystem impacts of this invasive species. Because *I. aquifolium* has tough, recalcitrant leaves, the species may also the potential to significantly alter ecosystem soil properties in some of the most productive forests in the northern hemisphere. We investigated soil characteristics underneath *I. aquifolium* canopies, and in adjacent paired locations without the invasive species, to determine the long term physical and chemical effects on the soil. Our chemical analysis focused on pH, carbon-nitrogen elemental analysis, mineralized nitrogen and phosphorus, extractable cations (K, Mg, Ca, Na), sulfate, soil respiration, and cation exchange capacity. We also investigated changes in soil moisture, organic matter content and bulk density. Our findings suggest heavy depositions of sulfur and organic matter associated with the presence of these trees ($p < 0.001$; $p < 0.031$ respectively) while soil moisture and pH were correlated with age of the trees ($p > 0.0074$; $p > 0.0031$ respectively). These results cumulatively suggest the potential ecosystem impacts of a novel invasive species in a temperate rainforest ecosystem.

Keywords

Ilex aquifolium; English Holly; Invasive; Soil Chemistry; Nutrient Cycling;

Introduction

Long term changes associated with the colonization of invasive species in native ecosystems are of growing concern, and invasion can have important impacts on soil characteristics [1]. Chemical changes in the soil may alter the available nutrient resources, affecting native plant's ecophysiological traits such as growth and nutrient allocation patterns [2] [3] [4]. For example, *Rosa rugosa*, a native of eastern Asia that has invaded northwest

Europe, has been shown to significantly alter the concentrations of mineralized potassium, magnesium, phosphorous, and nitrogen in soils [5]. A separate study in the lowland heaths of southern England showed an increase of 150-500% of phosphorous in areas heavily invaded by non-native species [6]. In both examples, the overall biomass of the invaded plots showed a dramatic increase in non-native biomass, outcompeting the native plants.

The invasive species *Ilex aquifolium* (English Holly), in Northwestern US forests, also has the potential to disrupt the delicate balance of ecosystem diversity and nutrient cycling [7]. Records show *I. aquifolium* was first introduced in 1869 and quickly became a widely cultivated ornamental plant throughout the Pacific Northwest [8]. A 2009 report by Seattle Urban Nature indicates that the average density of *I. aquifolium* is 328 stems per acre and currently constitutes more than 60% of all tree regeneration in coniferous forests within the city of Seattle [9]. *Ilex aquifolium* is a dioecious species, and has the ability to reproduce both sexually through seed dispersal, as well as asexually sprouting from branches that fall to the ground. A recent study suggests 78% of specimens propagated from existing vegetation, while 22% originated from seed, as berries do not develop until the tree is at least 15 years old [10]. As native fruiting plants are rarely found in winter, seed dispersal by birds represents a significant method for reproduction over large distances (Boersma et. al 2006). Although dispersed by several native birds, including: *B. cedrorum* (Cedar Waxwing), *T. migratorius* (American Robin), *Z. macroura* (Mourning Dove), *Poecile* spp. (Chickadees), and *Fringillidae* spp. (Finches), *T. migratorius* were documented to be the dominant feeder, with a 95% frequency rate compared to the other bird species [11]. Distribution by bird species allows the species to colonize undisturbed forest habitat. Physiologically, *I. aquifolium* may also be well adapted to penetrate coniferous forest understories due to its high shade tolerance [12] [13]. While its overall branching form may consist of a single stem or multiple stems depending on growing conditions, thick waxy leaves and a dense growth habit create a very low light environment beneath its canopy. With older individuals, a thick layer of slowly decomposing leaves can be found surrounding the tree, suggesting slow leaf decomposition with potential ecosystem implications.

Based on these characteristics, *I. aquifolium* has the capability of significantly affecting species composition and ecosystem function in native forests. However, as it was only recognized for its ecological invasiveness in the last decade, very little research has been done to quantify its impact on ecosystems. Here, we use a pair-wise sampling design, to examine a second growth forest heavily impacted by *I. aquifolium* in western Washington. Specifically, we use a paired sampling design to assess soil characteristics and nutrient cycling in both the presence, and absence of this invasive species.

Study Site

We examined a 380 ha forest located in the ~315 ha Evergreen State College Forest Reserve (**Figure 1**). Bordering the Salish Sea in Western Washington, our study site is a

second growth forest logged from 1937-39 using cable methods [14]. Supported by 100 cm of annual rainfall and a mean temperature of 10° C [15], the forest consists of ten distinct canopy types dominated by *Pseudotsuga menziesii* (Douglas Fir), *Tsuga heterophylla* (Western Hemlock), *Thuja plicata* (Western Red Cedar), *Acer macrophyllum* (Big Leaf Maple), and *Alnus rubra* (Red Alder). Dominant understory species are *Mahonia nervosa* (Dull Oregon Grape), *Polystichum munitum* (Sword Fern), and *Gaultheria shallon* (Salal). Using a pre-established network of permanent plots known as the Evergreen Ecological

Observation Network (EEON), we conducted an initial survey to quantify the status of *I. aquifolium* in the reserve [16]. Using the results from this survey, we created an interpolated heat map that reflects the density of *I. aquifolium* throughout the forest (**Figure 1**). As the southern portion of this forest seemed to be the most heavily invaded, as well as the least developed in terms of recreational use, it was chosen as the primary study site. Within this

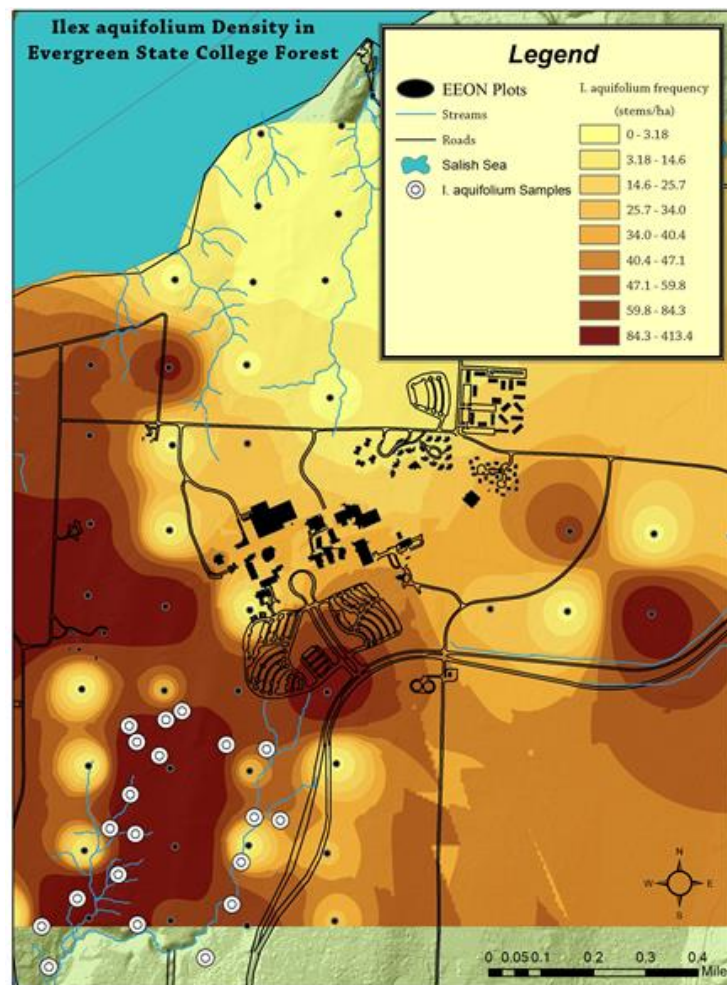


Figure 1. The Evergreen reserve located in Olympia WA. The heat map reflecting the density of *I. aquifolium* was created using inverse distance weighting interpolation in ESRI ArcGis. Samples for this study were concentrated in the southern portion of the forest.

area, twenty healthy, large, and isolated *I. aquifolium* trees were haphazardly selected (**Figure 1**). The twenty trees selected ranged in age from 25 – 55 years, reaching up to 23m in height and 23cm in diameter. In order to age the specimens, a Haglof increment borer was used to core the main trunk 15cm above the ground at all 20 plots. Cores were stored in slit straws until thoroughly dried to prevent warping and bending. Due to the density of the wood combined with diffuse porous properties, cores were mounted in boards trenched with a ½” router, sanded and polished. A high intensity lamp and 10x lens was then used to count rings.

Field Methods

At each of the twenty selected trees, we established a pair-wise sampling design (**Figure 2**). Samples collected for physical analysis (bulk density, organic matter and moisture content) were duplicated (two replicates at each sample distance from tree), while the samples for chemical analysis were triplicated (see **Figure 2**). Samples collected outside the canopy were taken 10m away from the main stem, where this distance was chosen based on the most extensive *I. aquifolium* canopy, roughly 5m in radius. Replicated sample sites were evenly separated by 60° where possible, though in some cases this was slightly altered in order to prevent the exterior samples from coming within 10m of another *I. aquifolium* tree. Soil cores were taken using 2” PVC to a depth of 10cm. Soil respiration was measured in situ using a LCpro+ with an open soil chamber, replicating methods presented in detail in Kirsch et al. (2011) between Jan 27th and March 26th, 2015. Briefly, measurements were taken at each soil location on bare soil for three minutes after allowing an equilibration period of the chamber with the soil (1 min). Measurements were taken every minute, and then

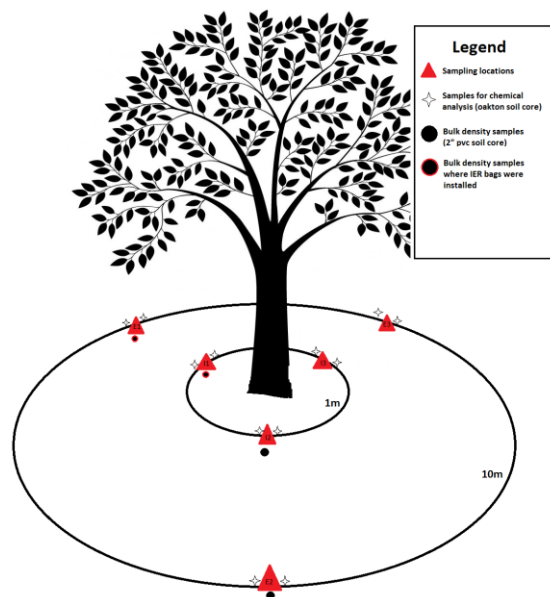


Figure 2. Pair-wise sampling design established at each *I. aquifolium* tree. Samples from the 1m radius were directly compared to samples at the 10m radius. Diagram not to scale.

the average of all three consecutive measurements was used for an estimate of soil CO₂ efflux. All measurements were taken between 1 pm and 5 pm each day to account for any potential diurnal trends in soil CO₂ efflux [14].

Laboratory Methods

Once soil samples were brought back to the lab, they were air dried in paper bags, run through 2mm sieves, and sorted into rocks, organic matter, and fine fraction soil. Subsamples were oven dried at 105°C and burned at 500°C to determine moisture and organic matter content respectively. To measure pH, we created a 50/50 solution of distilled water to fine fraction soil. The paste was stirred regularly for an hour then measured with an Oakton pH meter calibrated with a 7.0 pH standard. A thermal conductivity elemental analyzer was used to measure the C:N ratios in the soil samples (CHNS/O Analyzer Perkin Elmer 2400 II, PerkinElmer, Inc. Waltham, MA, USA). Orthophosphates were measured using a protocol adapted from Olsen & Summers (1982) using ammonium fluoride and hydrochloric acid [17]. Extractable potassium, magnesium, calcium, sodium and sulfur were determined using emission and absorption spectrometry. Cation exchange capacity was quantified using the method from Horneck et. al. 1989 using ammonium replacement [18]. Lastly we assessed mineralized nitrogen (NO₃⁻/NO₂⁻, NH₄⁺) using 2M KCl and cadmium reduction using a discrete autoanalyzer (AQ1 Discrete Analyzer, Seal Analytical Inc. Mequon, WI, USA), following methods from Keeny & Nelson 1982 [19].

Results

All tests were ran in JMP (SAS Institute 2014) either as pair-wise comparisons between the measurements taken 10m from the trunk and those taken beneath the canopy, or as bivariate regressions (F-test) where appropriate. The pair-wise comparisons allowed for a spatial analysis on soil impacts due to *I. aquifolium*. Significant comparisons were determined by analyzing the pair-wise difference by the global mean for each of the twenty specimens. Alternatively, the bivariate regressions were able to identify temporal trends over the 25 year range in specimen age. In these analyses, 1m and 10m samples were grouped together resulting in twice the sample size.

Physical Tests

While no spatial trends arose in soil moisture and bulk density from the pair-wise comparison, we see a pattern of increasing soil moisture as function of *I. aquifolium* age (**Figure 4**; $p > 0.007$). We did find a spatial trend in organic matter, with significantly more ($p < 0.03$) beneath the canopy of *I. aquifolium* than outside the canopy. We suspect this is due to a large deposition of thick and waxy leaves that slowly decompose when compared to the surrounding vegetation. This is particularly evident in older specimens that consistently develop dense and impenetrable canopies.

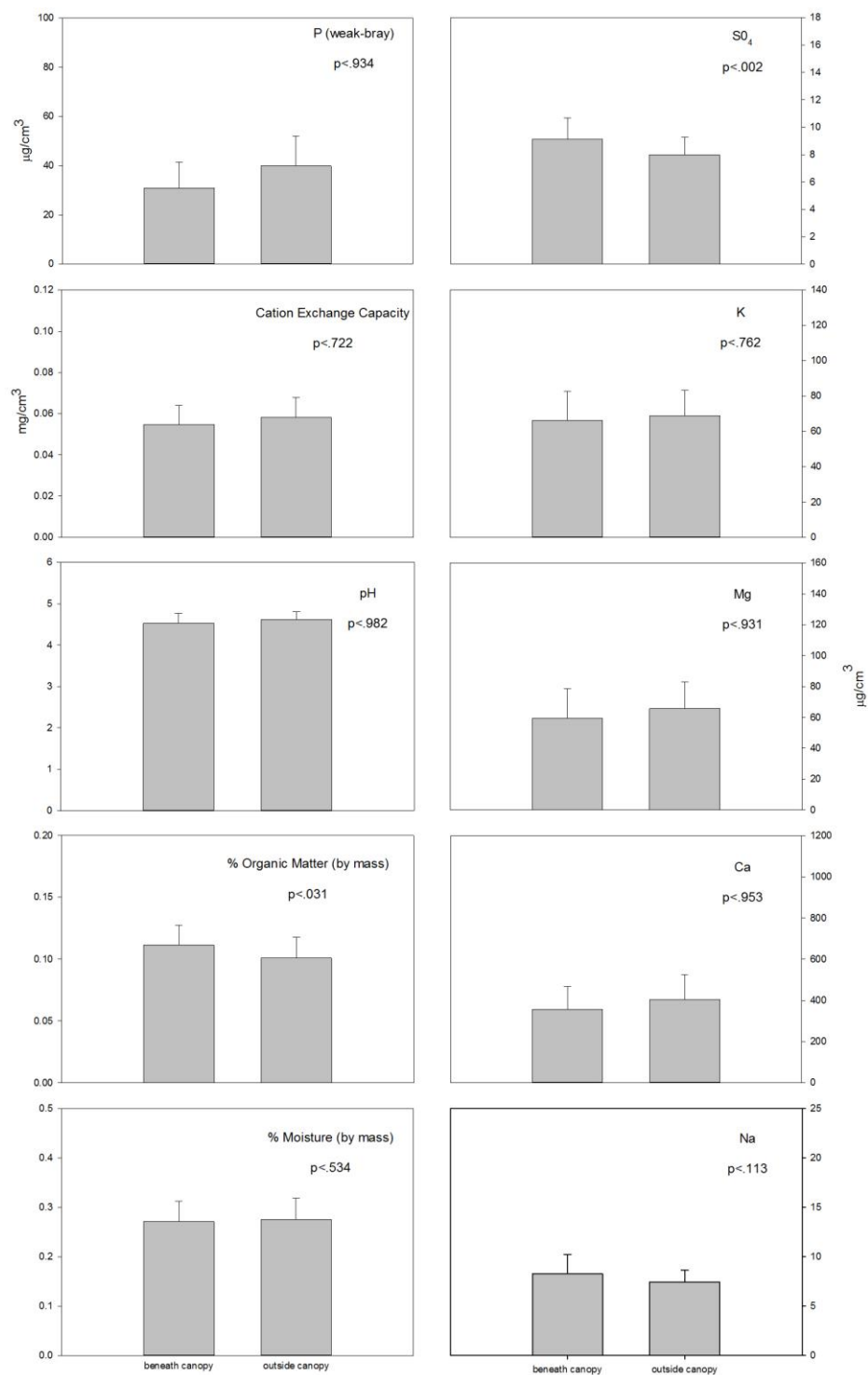


Figure 3. Average differences in soil characteristics between the 1m and 10m samples. Significance was determined using a comparison of the paired difference by the global mean. Error bars represent the 95% confidence interval.

Soil Respiration

Using the LCpro+ we averaged eight measurements beneath the canopy, and compared this with a second set taken at the 10m distance. In the pair-wise comparison, no significant differences were detected ($p < 0.239$). These findings suggest that the presence of *I. aquifolium* has not transformed the microbial community and soil biota in such a way as to affect soil respiration. Alternatively, it is possible that 10m was insufficient to detect any changes in soil respiration. Typically soil respiration is a function of soil moisture and to a lesser degree soil temperature [20]. However, despite a trend of increasing soil moisture, we see no consequences on soil respiration in our study. It is possible this is a seasonal effect. As measurements were collected in Jan-March (late winter – early spring), water was not a limiting factor. However, we couldn't eliminate the possibility of contrasting results corresponding to the dry months, and our data should be interpreted accordingly. Additionally, as these measurements were only taken at subset of the twenty sites, it is also possible our sample size was insufficient to detect trends in soil respiration.

Total Elemental Analysis; Mineralized Nitrogen & Phosphorus

Total nitrogen and carbon were measured using a PerkinElmer 2400 elemental analyzer (as described in methods above). The results from this test were reconciled with bulk density to achieve a better understanding of the carbon to nitrogen ratio at the different sites. These results were then compared to tests for mineralized forms of nitrogen (NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$) to determine the amounts of bound and available nutrients. In both our temporal and spatial analysis, we found no significant patterns associated with the C:N ratios or the concentration of mineralized nitrogen in the soil. Similar results were found in our analysis of orthophosphates.

Extractable Cations & Cation Exchange Capacity

Using mass spectrometry, we measured the concentrations of several common cations including Mg, Na, Ca, and K. Similar to the elemental analysis, we scaled up these results by using the bulk density of soil. Again, no significant trends arose. A summary of the p-values can be found in **Table 1**.

Sulfur

Here our results suggest a significant impact to the soil chemistry due to *I. aquifolium*. On average we found 15% more sulfur beneath the canopy when compared to the adjacent space 10m away ($p < 0.002$). While the source of this sulfur remains unknown, similar species in the *Ilex* genus have recently been found to excrete sulfur containing triterpenoid saponins from their roots [21]. We also found a temporal trend associated with sulfur, suggesting increasing concentrations in the soil with time $p > 0.043$. Our findings suggest a 50% increase in the concentration of sulfate since 1990, when the youngest specimens used in the study were first established.

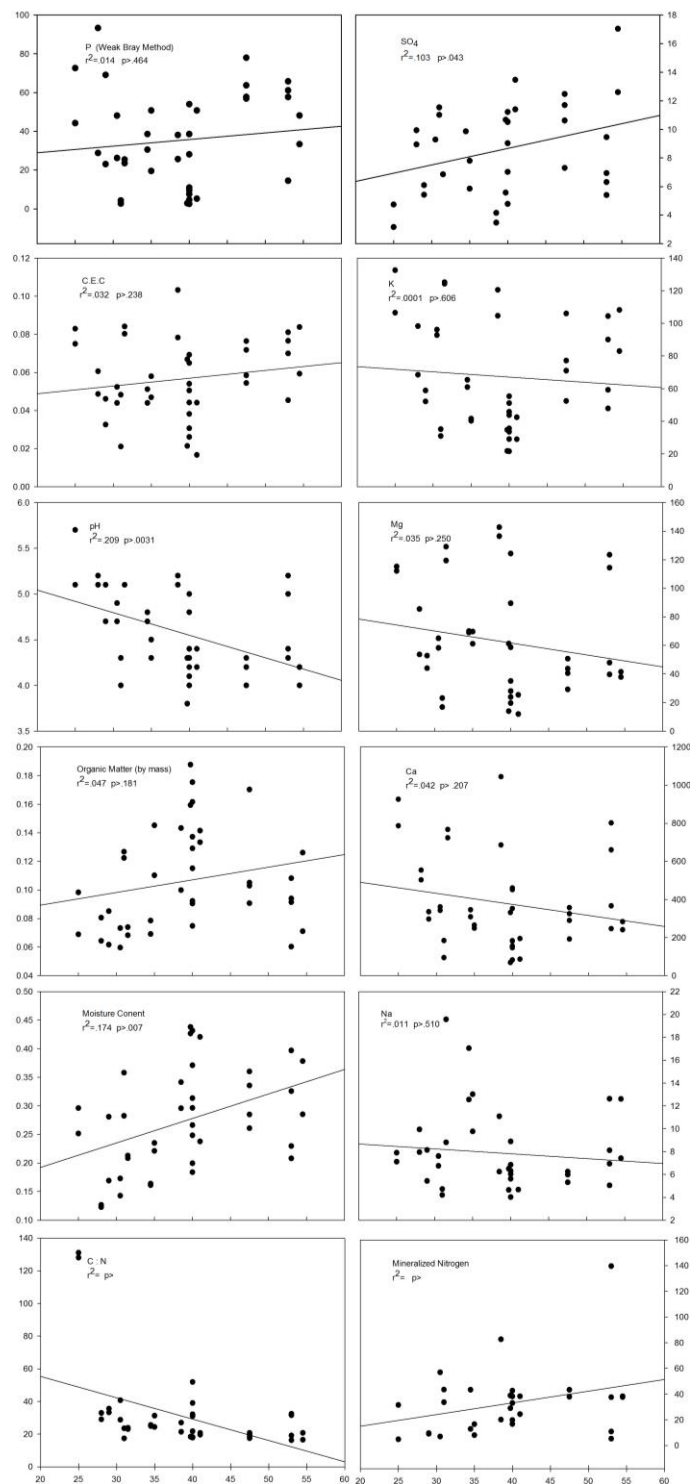


Figure 4. Bivariate regressions of the measured soil characteristics against the age of the specimens. 1m and 10m samples were grouped for this analysis resulting in twice the sample size. Significance was determined using an F-test.

Table 1. Summary of test statistics for both the spatial (T- test) and temporal (F-test) analysis of *I. aquifolium* on the soil.

	Moisture Content	Organic Matter	pH	P (weak-bray)	Mg	Ca	Na	C.E.C.	NCER	Sulphate-S	K	N-Min
Prob < t (by distance)	0.5340	0.0314	0.9822	0.9338	0.9311	0.9531	0.1132	0.7222	0.2387	0.0016	0.7621	0.4430
Prob>F (by age)	0.0074	0.1805	0.0031	0.4638	0.2500	0.2070	0.5102	0.2831	-	0.0430	0.6064	0.0892
F Ratio	7.9963	1.8613	10.0145	0.5478	1.3645	1.6478	0.4420	1.1853	-	4.3837	0.2698	3.0800
Rsquare	0.1738	0.0466	0.2085	0.0142	0.0346	0.0415	0.0114	0.0320	-	0.1034	0.0007	0.0850

pH

While we did not find any spatial trends in the pair-wise comparison, we see a strong relationship of decreasing soil pH in relation to *I. aquifolium* age ($p > 0.003$) (**Figure 4**). Over the 25 year range in specimens, we see a ten-fold drop in pH from 5.0 to 4.0. As elemental sulfur is commonly used to lower the pH of soils, we investigated the possible correlation between the concentration of sulfate and soil pH (**Figure 5**). Our bivariate analysis revealed a very strong relationship ($p > 0.0001$) indicating that sulfate concentration was an excellent predictor of soil pH. While the mean pH across all soil samples was 4.5, previous measurements of soil pH from the EEON network suggest a higher average value of 5.5. From this we speculate that *I. aquifolium* is impacting soil pH through sulfur deposition, though a distance of 10m was insufficient to detect any spatial patterns.

Discussion

This study supports the scarcely discussed idea that *I. aquifolium* is impacting the soil of Pacific Northwest forests. Our findings suggest *I. aquifolium* acts as a significant source of organic matter and sulfur. These trends were accompanied by a 10-fold decrease in soil pH over a 25 year period. While sulfur appears to be one of the primary drivers of this acidification, its source and form remain unstudied. As our initial surveys of the study site suggest densities of *I. aquifolium* over 300 stems/ha, we suspect that such concentrations help to facilitate measurable changes in the soil chemistry over short periods of time. Moreover, these trends are likely exponential, closely mirroring the rate of colonization. As these transformations broaden their horizons, native species are likely to be displaced, unable to adapt to the rapidly changing soil conditions. However, this will require close monitoring over the years to come.

The majority of the largest specimens occurred in wetlands or on the periphery of drainages. These sites were commonly dominated by the deciduous *Alnus rubra* resulting in little competition for light during the winter months, possibly one of the factors affecting the vigor of the largest specimens. The species appears to be extremely resilient as individuals were commonly observed to be crushed but flourishing beneath fallen *A. rubra*. Furthermore, over the course of the study we were unable to locate or identify any dead or visibly unhealthy individuals. The oldest specimens used in the study were 55 years of age, consistent with the last logging of the study site. However, as *I. aquifolium*

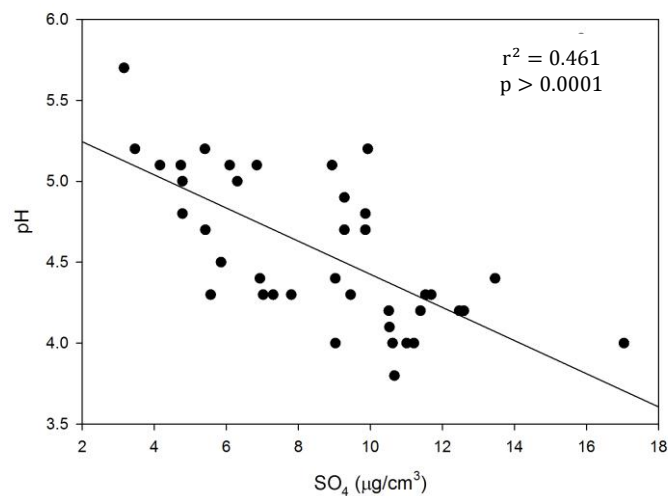


Figure 5. Bivariate regression of sulphate to pH. With a significant p-value of $p > 0.0001$, we suspect that sulphate is the primary driver of soil acidification

lives up to 250 years in its home range, we find support for sustaining long-term soil transformations [22].

While these findings complement a small but growing body of research focusing on *I. aquifolium* in the Northwest, more research is needed in order to draw attention to the magnitude of this invasion [7] [10]-[12]. As *I. aquifolium* has broken out of the lag phase of invasive colonization and begun to reproduce at exponential rates, our findings suggest serious implications for the health of second growth forests of the Pacific Northwest [10].

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