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 ERDUGVWUHQFKHGZLWKDURWHUVDQGHGDQGSROLVKHG. 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. 6RLOFRUHVZHUHWDNHQXVLQJ 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

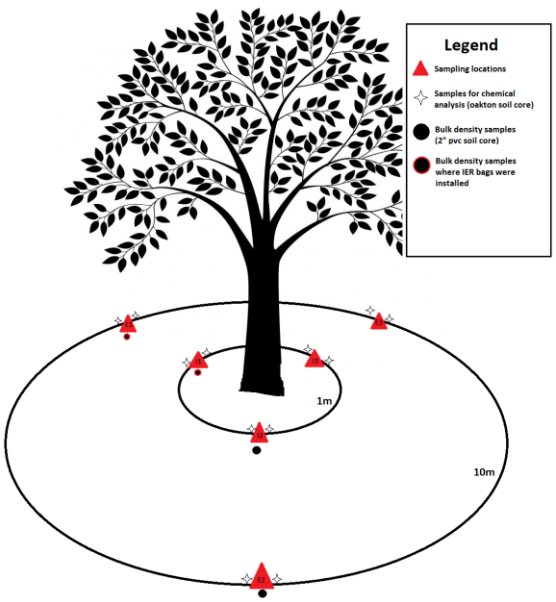

Figure 2. Pair-wise sampling design established at each *I. aquifolium* tree. Samples from the 1m radius were directly compared to samples at the 10m radius. Diagram not to scale.

the average of all three consecutive measurements was used for an estimate of soil CO_2 efflux. All measurements were taken between 1 pm and 5 pm each day to account for any potential diurnal trends in soil CO_2 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 (NO_3^-/NO_2^-, NH_4^+) 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.

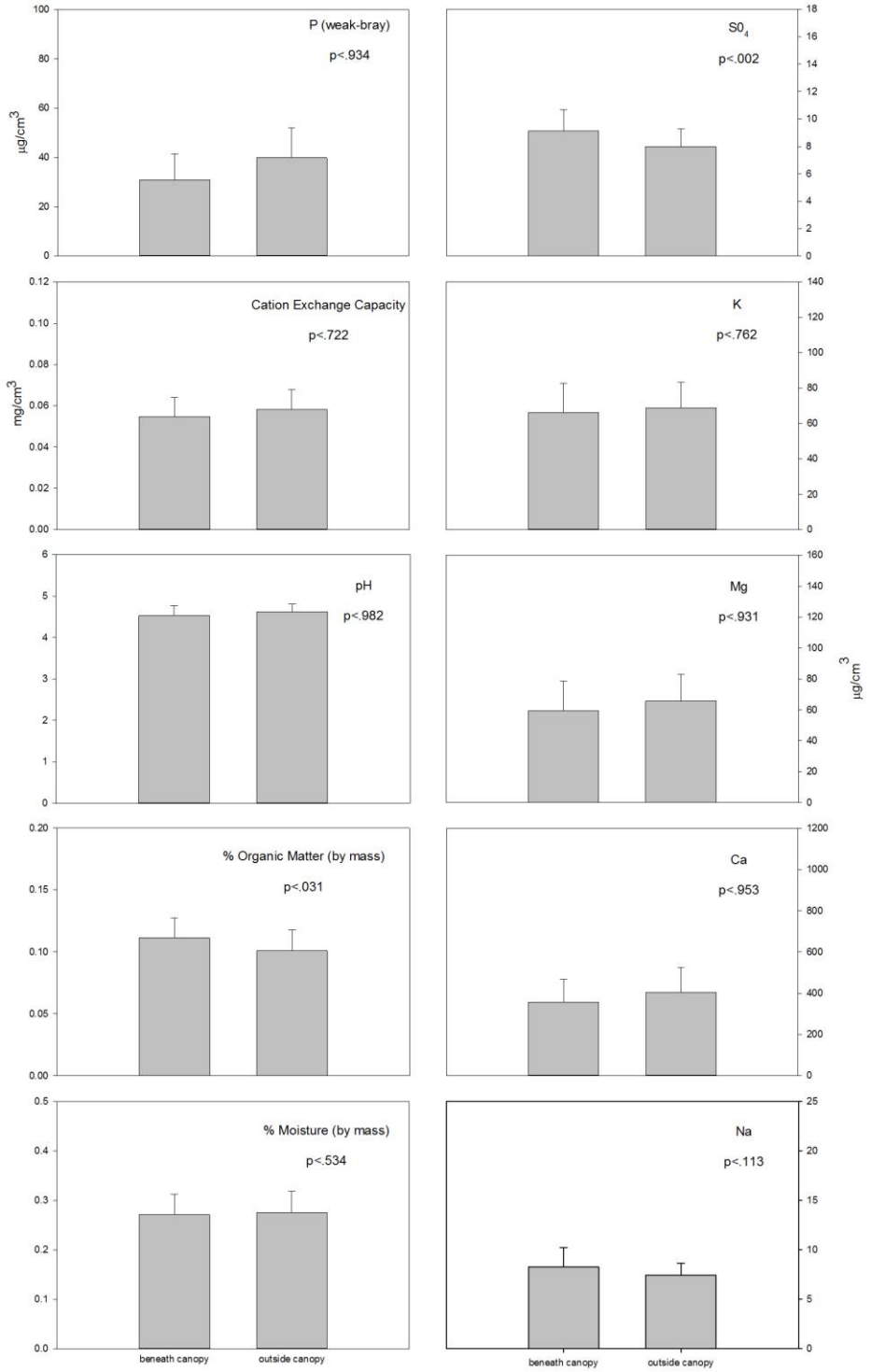

Figure 3. Average differences in soil characteristics between the 1m and 10m samples. Significance was determined using a comparison of the paired difference by the global mean. Error bars represent the 95% confidence interval.

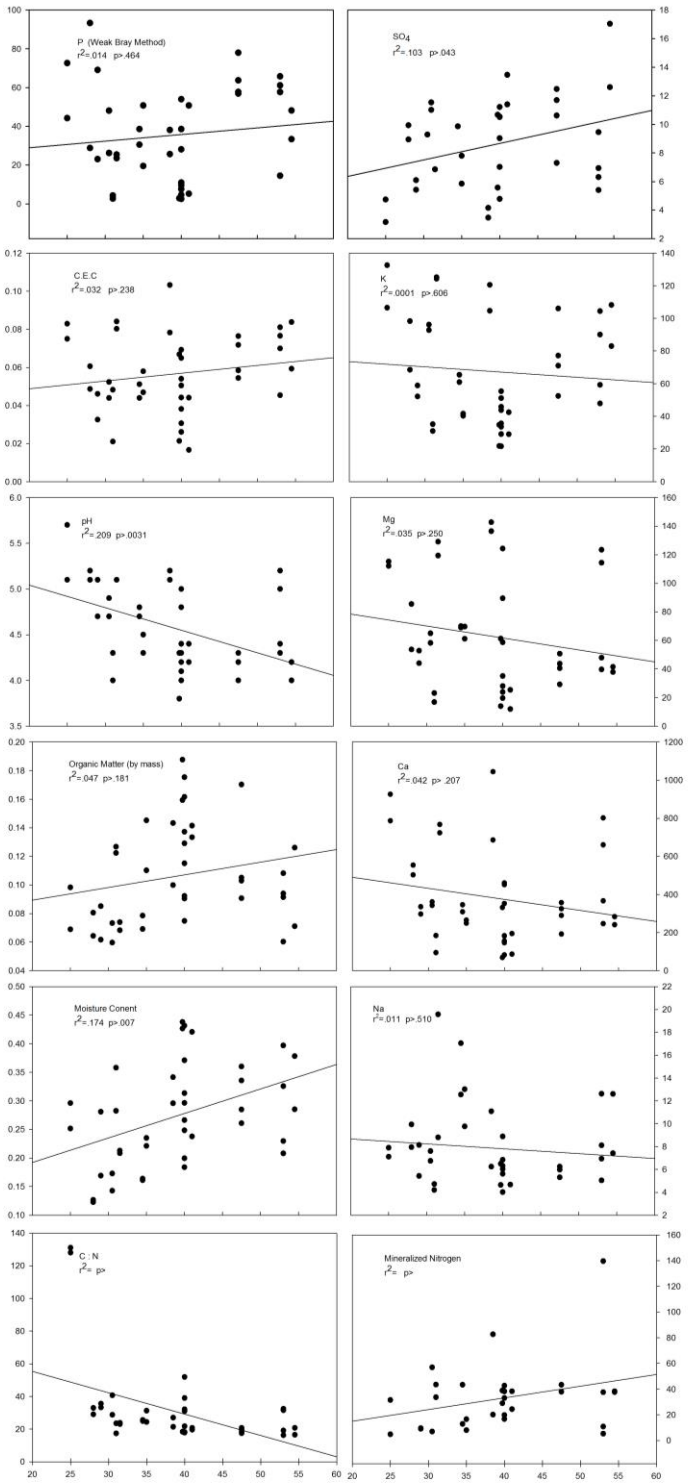

Figure 4. Bivariate regressions of the measured soil characteristics against the age of the specimens. 1m and 10m samples were grouped for this analysis resulting in twice the sample size. Significance was determined using an F-test.

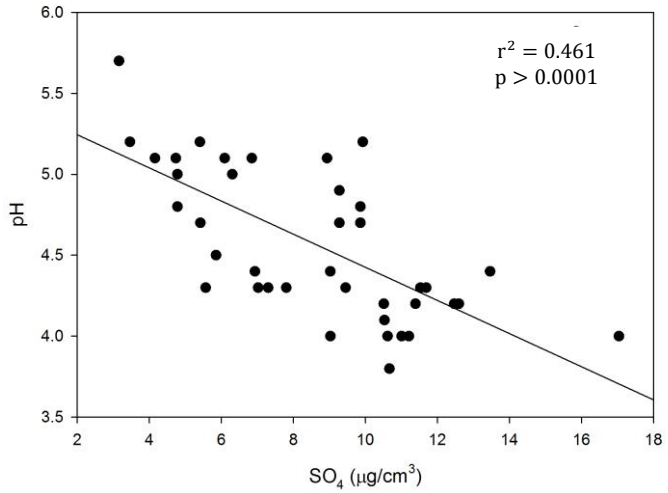

Figure 5. Bivariate regression of sulphate to pH. With a significant p-value of p>0.0001, we suspect that sulphate is the primary driver of soil acidification

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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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