COMPARISON OF NATURAL AND ANTHROPOGENIC EFFECTS ON
NEARSHORE PERIPHYTON IN LAKE CRESCENT, WASHINGTON STATE

by

SIMEON FRANCIS REDBERG

A THESIS

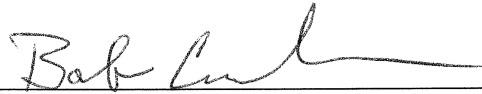submitted to

WALLA WALLA UNIVERSITY

In partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 29, 2012

This thesis for the Master of Science degree

has been approved by the Department of Biological Sciences

and the Office of Graduate Studies
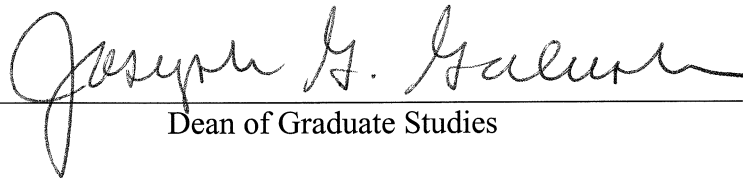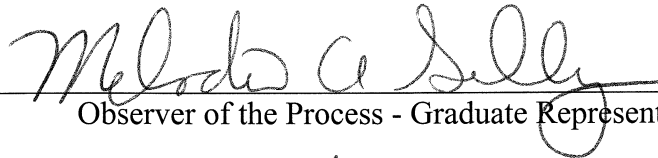
Walla Walla University

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Major Professor

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Committee Member

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Committee Member

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Committee Member

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Dean of Graduate Studies

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ABSTRACT

Periphyton on natural and artificial substrates were collected from four developed and four paired undeveloped sites along the north shore of Lake Crescent, Washington to determine if development or natural factors had a greater impact on periphyton growth and periphyton algal community composition. Ash-free dry weight, chlorophyll a, and the proportion of filamentous green algae were higher at sites with a gradual offshore slope and at sites with greater alder cover, while diatoms were lower at those same sites. Conifer cover had little impact, only affecting other algae which were more abundant at sites with less conifer cover. Development had little, if any, impact on periphyton growth or community composition. I conclude that at this time in the lake natural factors such as shore topography and alder cover have a stronger influence on periphyton growth than do anthropogenic factors such as the septic tanks associated with developed sites.

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INTRODUCTION

Deep oligotrophic lakes with low levels of nutrients in the water column such as Lake Tahoe (Goldman, 1988) and Lake Crescent in Washington State (Meyer and Fradkin, 2002) are often associated with crystal clear waters and provide amazing recreational opportunities. However, human activity often impacts the balance of nutrients in lakes. Runoff from fertilizers and untreated sewage creates a potentially large eutrophication impact on lake ecosystems through addition of nutrients to the system and altering the natural cycling of phosphorus and nitrogen levels (Horne and Goldman, 1994). The increase in nutrients can cause substantial algal blooms which can release toxins that negatively impact zooplankton as well as larger inhabitants such as fish. In extreme cases large algal blooms are followed by crashes which have negative effects of their own such as oxygen depletion, smell, and more release of toxins. In deep oligotrophic lakes such as Lake Crescent the water can lose its clarity lessening light penetration and triggering an overall change in the food web dynamics of the lake (Vadeboncoeur and Jeppesen, 2003). Efforts to reverse eutrophication due to excess nutrients have focused on better water treatment, the reduced use of fertilizers and phosphates in detergents, as well as efforts to remove existing phosphorus and nitrogen pools. Responses to such efforts have often been slow, although many success stories exist (Horne and Goldman, 1994).

Studying the early stages of eutrophication of deep oligotrophic lakes has proven difficult because nutrient levels in the open waters of these lakes often barely reach standard detectable limits even though important changes may be happening (Goldman, 1988, Meyer and Fradkin, 2002). However, Vadeboncoeur and Jeppeson (2003) showed

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that even in steep-sided deep lakes periphyton production (algal growth on the bottom near the lake margins) was a substantial portion of the entire lake's productivity. In addition, periphyton are important to whole-lake nitrogen cycling (Reuter et al., 1986) Also, because the periphyton grows along the water-substrate interface, many nutrients entering by way of stream or ground water may be picked up rather quickly by the periphyton (Loeb and Goldman, 1979, Biggs, 1989) rather than entering the pelagic zone. Consequently, periphyton growth may be an especially important indication of changes occurring in deep lakes (Biggs, 1989).

Using periphyton growth and community composition, Rosenberger et al. (2008) determined that periphyton biomass was higher at developed sites than at undeveloped sites in Lake Crescent and that species of algae associated with eutrophication such as filamentous green algae were more common at developed sites. However, they reported that they could not discriminate between developed and undeveloped sites in regard to shifts in $\delta^{15}N$ which are usually associated with human sewage and fertilizers. They also noted that there were several other differences between the developed and undeveloped sites in their study, perhaps the most significant being tree community composition (Rosenberger et al., 2008). They found that while tree density was not significantly different between developed and undeveloped sites, tree species and diameters were significantly different with developed sites having more alders and trees with greater diameters. The study also did not compare the similarity of their developed and undeveloped sites in terms of substrate type, shoreline slope, or direction of exposure which also may significantly affect the extent of periphyton growth and periphyton community composition (Vadeboncoeur and Jeppesen, 2003). The findings of

Rosenberger et al. (2008) thus suggest that further focused study looking in detail at these issues would be beneficial.

The purpose of this study was to reexamine and compare the periphyton growth and community composition on natural and artificial substrates between near shore developed and undeveloped sites in Lake Crescent. The developed and undeveloped sites were as closely matched as possible in regard to tree coverage and species type as well as exposure, substrate type, and offshore slope. Periphyton growth, biomass, and community composition on the natural and artificial substrates were compared among sites to determine whether the observed differences were primarily due to human-associated eutrophication or whether they could also be a result of other, natural factors.

Study Site

Lake Crescent (Figure 1) is a deep, clear lake located 24 km west of Port Angeles, WA. Although it is almost wholly within the boundary of Olympic National Park, a number of private residences, on land privately owned before the park was formed, exist along the shoreline. Lake Crescent is a deep, steep-sided lake which covers 18.8 km^2 and has a maximum depth of 190 m. It is fed by several water sources with Barnes Creek being the most important and has one outlet, the Lyre River, near the northeast corner. The ridges that surround Lake Crescent and define the narrow watershed are quite steep and rocky and are covered mostly with conifers, with Douglas fir and western hemlock being the predominant species. Red alder and big leaf maple and other deciduous species also grow along the lake (Meyer and Fradkin, 2002).
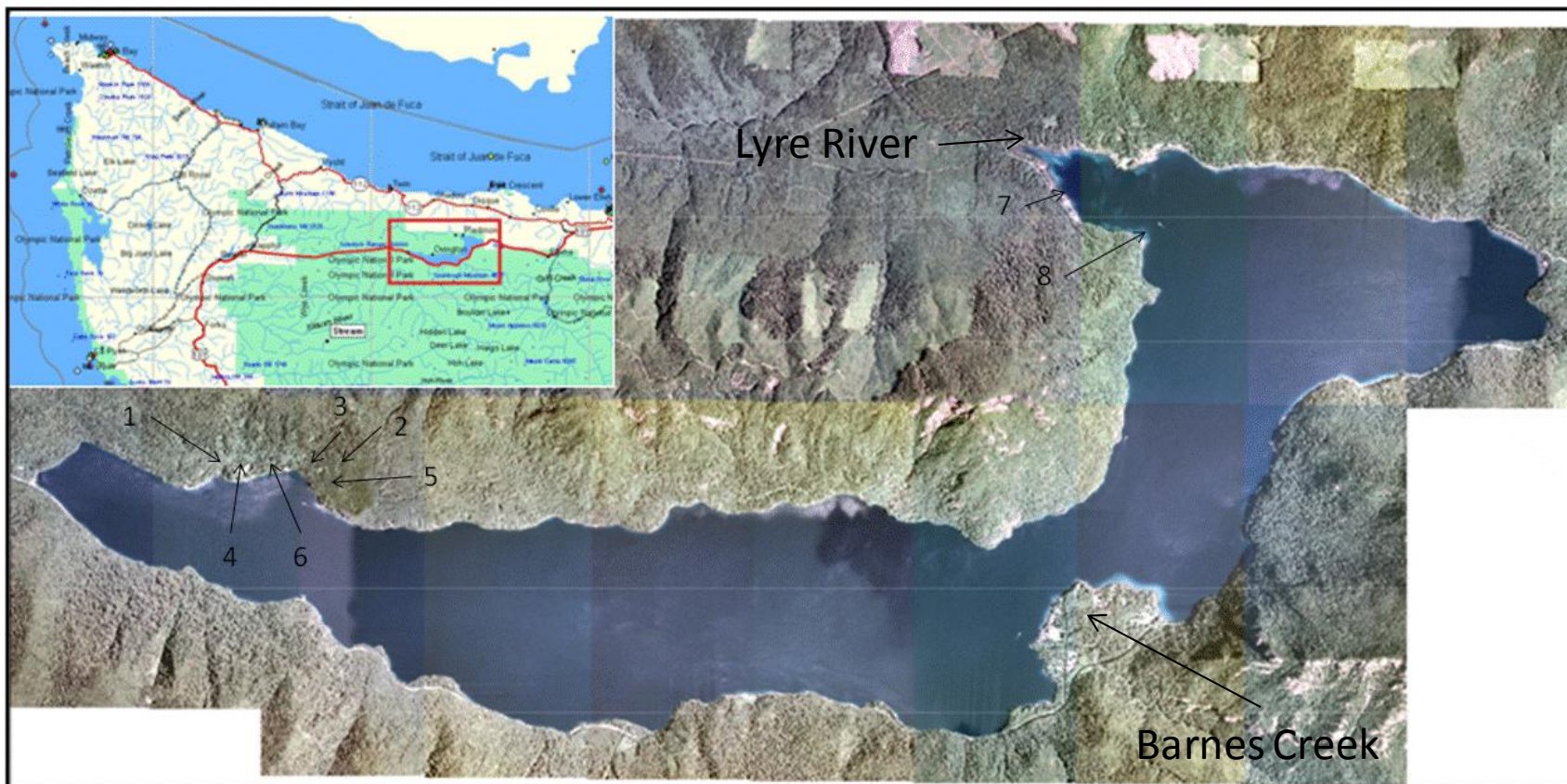
Figure 1. Map showing location of Crescent Lake on the Olympic Peninsula and study sites (See Table 1).

In 2002 there were 121 houses or cabins, 3 resorts, 1 store, and a campground located along the lake shore. 15.7% of the residences are occupied year round (Meyer and Fradkin, 2002). One hundred and six tracts of land that lie along the lake are privately owned (Meyer and Fradkin, 2002).

Lake Crescent was reported to be ultra-oligotrophic in 1989 and in 1998 (Rector and Hallock, 1991, Washington State Department of Ecology, 1998). In the 1998 survey the lake was so clear that the Secchi disk line was not long enough to take readings. Meyer and Fradkin (2002) report that from 1994-2001 total phosphorus and total nitrogen levels were below detection limits or barely detectable, and that dissolved oxygen levels were consistently high. Unlike most temperate lakes, Lake Crescent shows signs of possibly being nitrogen limited (Washington State Department of Ecology, 1998, Meyer and Fradkin, 2002). Although the lake has historically been remarkably clear and mostly free of algae, Meyer and Fradkin (2002) reported growth of algal taxa which are associated with eutrophication in some areas. They suggest this increase "may be related to nutrient enrichment from older potentially failing septic systems and sewage treatment plants along the lakeshore."

METHODS

Sampling Sites

The main focus of this study was to determine whether anthropogenic or other natural factors had the greater impact on nearshore periphyton growth and community composition. Four developed sites (Figure 1, Table 1) along the shore were chosen, including three sites currently used for housing and one site that was occupied until recently. For a site to qualify as "developed" it had to have a residence with a septic tank or system, a partial clearing of trees, and preferably a lawn. For logistic purposes, all the developed sites chosen were owned by Olympic National Park. None were within 100 m of the input of a stream in order to eliminate stream influence as a confounding variable. A survey of relevant characteristics both onshore and offshore was conducted in order to select four nearby matching undeveloped sites (Figure 1, Table 1). For a site to qualify as a matching "undeveloped" site, it had to have no currently or recently occupied buildings or lawns, not be within 100 m of a stream, and preferably be within 2 km of the developed site with which it was designated a match. Onshore characteristics had to also be comparable. Undeveloped sites were to have alder cover within 25% of that found on the paired developed site, and within 50% cover of other tree categories. The target for wave exposure and southern exposure was to be within 20° at matched sites. Depth at 10 m offshore was to be within 1 m between matched sites, and the substrate mix on the lake bottom was to be similar.

Onshore characteristics that were measured at each site included tree cover, the direction of the shoreline's exposure to waves, and exposure to the southern sky (which is an estimate of the amount of sun the site is exposed to). Onshore tree cover percent was

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Table 1 Physical and biotic characteristics of each site and t-test comparison of conifer and alder cover from developed and undeveloped sites									
Site	Site #	UTM (Zone 10, WGS 84)	Depth 10m Offshore (m)	Slope Category	Conifer Cover (m^2)	Cover Level	Alder Cover	Cover Level	Development Status
Tinkham House	1	0433338, 5324308	4.6	Steep	420.2	High	131.7	Low	Developed
Tinkham Control	2	0434092, 5324233	3	Shallow	378.6	High	171.9	High	Undeveloped
Chalet	3	0433928, 5324336	6.1	Steep	140.6	Low	256.3	High	Developed
Chalet Control	4	0433829, 5324331	-	Steep	350.5	High	58.5	Low	Undeveloped
Octagonal House	5	0434224, 5324029	2.1	Gradual	87.0	Low	133.1	Low	Developed
Octagonal Control	6	0434457, 5323900	1.4	Gradual	256.0	High	75.1	Low	Undeveloped
ONP Lab	7	0440461, 5326800	0.6	Gradual	209.9	Low	257.1	High	Developed
ONP Control	8	0441217, 5326332	-	Gradual	170.1	Low	136.1	Low	Undeveloped
t-test Results	Mean (m^2)	t	df	P					
Conifer									
Developed	214.4								
Undeveloped	288.8	-0.335	6	0.749					
Alder									
Developed	194.6								
Undeveloped	110.4	0.263	6	0.801					

originally determined using a spherical crown densiometer every 10 m along a 50

m transect that was placed 10 m from, and parallel to, the shoreline during the late

summer and winter. However, values for tree cover obtained using this method were

deemed unreliable due to the inability to produce consistent results. Therefore, tree

canopy area was determined again during the spring by measuring the diameter of the

canopy of every tree which had a trunk within 10 m from the shoreline over a distance of

50 m of shore at each site. Canopy area was calculated for each tree by multiplying the

diameter by πr^2, which assumed that the canopies were approximately circular. These

spring measurements were the values used in subsequent analysis.

Total tree canopy cover was divided into three categories: alder cover, conifer

cover, and other deciduous cover. For each of these three types of tree cover each site

was divided into either a "high" cover or "low" cover category. Sites were determined to

be in the "high" tree cover level when the area of the type of tree cover being considered

was higher than the average of all the sites for the same tree cover type. If tree cover was

below average, the sites were designated as "low" tree cover (Table 1).

Wave exposure was determined by measuring the compass direction normal to the

shoreline. Average exposure of the site to the southern sky was determined by measuring

the height in degrees of the southern horizon directly to the south, 45° to the southwest,

and 45° to the southeast and averaging these values.

For each site the depth was measured 10 m offshore. These measurements were

used to divide the sites into those with "gradual" and "steep" offshore slopes. Sites with

"steep" offshore slopes were sites that had a greater depth at 10 m than the average depth

off all the sites. The characteristics of the lake bottom (approximate proportions of silt,

sand, gravel, and rock) were also noted in order to attempt to match developed and undeveloped sites.

Substrate Sampling Protocol

Samples were collected during three seasons for this study. The first collection was made between August 30-September 1, 2010 (late summer). A second collection was made between December 13-15, 2010 (winter), and the third and final collection was made March 14-16, 2011 (spring). For the study, periphyton was collected from both natural substrates and artificial substrates at each site. Artificial substrates were made using 20 x 30 cm glass plates glued to concrete backing boards (Figure 2). The plates had a matrix of nine 5 x 5cm squares drawn on them, leaving a 2.5 cm perimeter to minimize "edge effects." Six of these plates were placed at each site, three at ~0.5 m, and three at ~1 m depth. Natural substrates used were undisturbed, naturally occurring rocks with at least a 25 cm^2 upper surface area which were already in place at each site. Three of these natural substrate samples were taken from ~0.5m and three were taken from ~1 m at each site during each season to match the depth characteristics of the artificial substrates.

Natural substrates at the sites included both soft sediment and rocks but for greatest comparability to the artificial substrates in this study only the rocks were sampled. During sampling of both natural and artificial substrates, the substrates were brought to the surface and an area of 5 x 5 cm from the upper, exposed surface was scraped clean with a razor blade and placed in a 50 ml centrifuge tube covered with foil to keep it dark. These were placed on ice and transported back to the biology department

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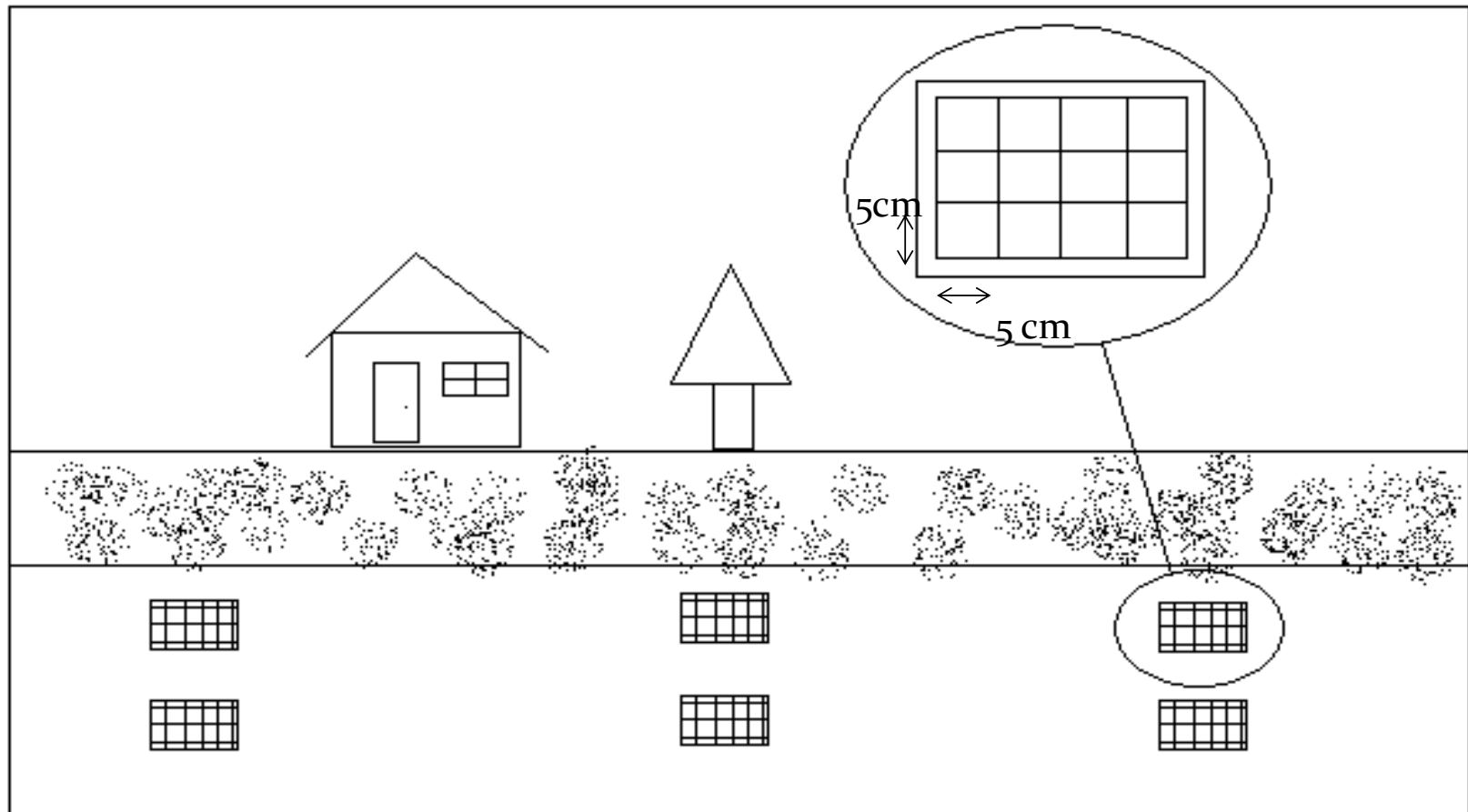
Figure 2. Diagram of artificial substrates and distribution of substrates at a study site. Each plate was made of a plate of glass glued to concrete board with twelve 5 x 5 cm squares drawn on them and at least 2 cm around the squares to avoid the "edge effect." Six plates were placed at each site with three at ~0.5 m and three at ~1 m.

lab at Walla Walla University. After sampling a rock for natural substrate, the rock was thrown out into deep water to prevent it from being sampled during any subsequent season.

A total of six natural substrate samples were collected from each site during the late summer, winter, and spring sampling seasons. In the lab, each periphyton sample scraped from a natural substrate was brought to 50 ml using distilled water, then after being stirred vigorously, was divided into 3 subsamples to be tested for ash-free dry weight (AFDW), chlorophyll a, and algal community composition. The artificial substrates were not sampled in the late summer since they had just been put in place. They were, however, sampled in winter and spring along with the natural substrates. With the artificial substrates, three separate 5 x 5 cm replicates were taken from each of the six sample plates at each site. These replicates were used separately for AFDW, chlorophyll a, and community composition analysis. In addition, during the spring sampling an additional 5 x 5 cm replicate was taken from a square which had been scraped the previous (winter) season. This replicate, designated the "resample" was analyzed for algal periphyton growth in order to compare algal growth on freshly exposed substrates to that on older substrates.

Macroinvertebrate Sampling Protocol

A 0.5 x 0.5 m area of relatively soft or small substrate on the bottom was chosen at each site in order to collect macroinvertebrates during each season sampled. Gravel, sand, and the debris from the top several cm of each area was removed and brushed over

a kick net by hand to dislodge any macroinvertebrates. Samples were then placed in a 50 ml vial and preserved using 70% ethanol.

Analysis Protocol

Periphyton scraped from both natural and artificial substrates was used for ash-free dry weight (AFDW) analysis, chlorophyll a analysis, and to determine algal community composition. AFDW, which is a measurement of total organic matter, was determined according to the protocol in Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). Six natural substrate samples and 6 artificial substrate samples from each site were analyzed for each season. Briefly, AFDW samples were placed in pre-weighed aluminum cups and dried to the nearest 0.001 g constant weight at 105° C. Samples were then placed in a muffle furnace and ignited at 500° C for one hour after reaching 500° C to oxidize away any organic material. Samples were then rewetted using distilled water and dried to a constant weight. AFDW was determined by using the equation $(W_i-W_c)-(W_f-Wc)$ where W_i is the initial dry weight, W_c is the weight of the cup, and W_f is the final constant weight after ignition. Since the ignition protocol is sufficient to completely oxidize the organic material but not inorganic minerals to carbon dioxide and water, AFDW is a measure of the mass of organic material present.

Chlorophyll a was determined spectrophotometrically according to Standard Methods (Rice et al., 2012) on six samples from each substrate type during each season for each site. Briefly, each sample was homogenized in 90% acetone to extract the pigments and absorbencies were taken at 750, 665, and 664 nm. Samples were then acidified using HCL and read again. Chlorophyll a values were calculated using the

equation chl a(mg/L)=(26.7)(A664c-A665c)(v)/(A)(Z) where 26.7 is a constant, A664 is

the absorbance at 664nm corrected for turbidity, A665c is the absorbance at 665nm after

acidification and corrected for turbidity, v is the volume of the extract in liters , A is the

area scraped in square meters, and Z is the length of the light path through the cuvette in

cm (in this case Z=1, Rice et al., 2012).

The autotrophic index (AI), which is a measurement of how much of the organic

material present is actually photosynthetic material, was calculated by dividing the

AFDW by chlorophyll a content. Note that a high AI represents sites at which less of the

organic material present is actually photosynthetic material (in other words, AI varies

inversely with proportion of photosynthetic material).

In order to determine periphyton community composition, algae cells were

counted similar to the approach used in Rosenberger et al. (2008). Briefly, six replicates

from each substrate type from each site during each season were preserved in Lugol's

solution until they could be counted. In addition, during the spring, "recount" samples

were taken from each of the artificial substrates which had been scraped during the winter

and recounts were made to determine spring algal growth on newly exposed surfaces.

Samples were homogenized using a glass/glass tissue grinder and viewed on a

hemocytometer using a light microscope at 400X. Two hundred cells were counted from

each sample and divided into the categories of filamentous green algae, cyanobacteria,

diatoms, and other algae using Freshwater Algae of North America (Wehr and Sheath,

2003) to identify the algae type.

Statistical Analysis

After site characteristics were measured, it was decided that only those characteristics with the most variation among sites would be used. After covariates were removed, characteristics that were used by Rosenberger et al. (2008) were also considered. This resulted in choosing the slope off shore, the development status, the level of conifer cover, and the level of alder cover as the independents by which the sites were to be compared. The other independent variables were eliminated because they showed little or no correlation with periphyton growth or strongly covaried with another of the independent variables. The samples gathered at 0.5 m and 1 m depth were not significantly different from one another so data from all six samples of each substrate type from each site were combined into one value for analysis.

Data from different seasons and substrate types were transformed separately using the Box-Cox transformation to optimally normalize the data, then were analyzed using SPSS ® 12 general linear model (GLM) tests using offshore slope, conifer tree cover level, alder tree cover level, and development status as the independent variables. Dependent variables were AFDW, chlorophyll a content, AI, and counts of filamentous green algae, cyanobacteria, diatoms, and other algae. Periphyton cell type ratios were arcsine transformed before analysis. Box-Cox transformations were done separately by season except when seasons were being compared with one another, in which case the transformation was made for data from all seasons combined. Seasonal comparisons were done using a one-way ANOVA on natural substrates and an independent samples t-test for artificial substrates. Comparisons between natural and artificial substrates were also done with independent samples t-tests. A canonical correlation analysis was also done

using SPSS ® 12 to determine what significant multivariate correlations, if any, existed

between the independent and dependent variables (canonical correlation is a type of

multivariate analysis that determines how much of the variance in one set of variables can

be explained by variance in another set of variables along one or more axes).

RESULTS

Site Characteristics

Locating suitable developed sites proved to be more difficult than anticipated. Since many of the privately owned sites were seasonal residences or the owners were hard to contact, it became impractical to use privately owned developed sites. The sites that were used belonged to the Olympic National Park and all were on the north shore. Preliminary matching of developed and control sites also proved more difficult than anticipated due to the limited number of appropriate sites available. Although the undeveloped sites chosen as controls were visibly similar to the developed sites, after measurements of the onshore characteristics it was determined that not all the control sites were as similar in all respects to their matching developed site as had been originally planned. However, average characteristics of developed and undeveloped sites as a whole were not significantly different in offshore slope, conifer cover, or alder cover (Table 1). Other characteristics such as direction of wave exposure and amount of exposure to sunlight from the southern horizon were shown to have little variation across all sites due to the fact they were all on the north shore and were therefore not included as variables in further statistical analysis. Total tree cover was not further studied since it was mostly made up of alder and conifer cover. Other deciduous cover (not alder) was not further studied because it showed little variation among sites.

Seasonal Observations of Periphyton Growth

On natural substrates there was a visual but not statistically significant increase in ash-free dry weight (AFDW) from the late summer to the winter sampling periods. The

slight increase from winter to spring was not significant on natural substrates but was on artificial substrates (Figure 3, Table 2). Chlorophyll a on natural substrates showed a more delayed increase, remaining low in late summer and winter and only increasing visually but not significantly in the spring (Figure 4). On artificial substrates there was a significant increase in chlorophyll a from the winter to the spring however (Figure 4). Due to the delayed increase in chlorophyll a but not AFDW on natural substrates the autotrophic index (AI) increased significantly from the later summer to winter and decreased slightly but not significantly from winter to spring (Figure 5). On artificial substrates the trend was different with the AI increasing significantly from the winter to the spring (Figure 5).

The most common types of algae by cell count on all substrates were filamentous green algae, cyanobacteria, and diatoms (Figure 6). Filamentous green algae were at lowest abundance in the winter at about 14% of total algal cells, but increased in abundance to 22% of total cells in spring and 44% of total cells by late summer. Diatoms were always abundant but peaked at 61% of total cell counts in the winter. Cyanobacteria were usually less prevalent than were filamentous green algae and diatoms. Cyanobacteria abundance was lowest in the late summer, increasing in the winter, and decreasing again in the spring. Other algae made up only a small percentage of the total cell counts during each season, but were lowest in the late summer and highest in the spring (Figure 6).
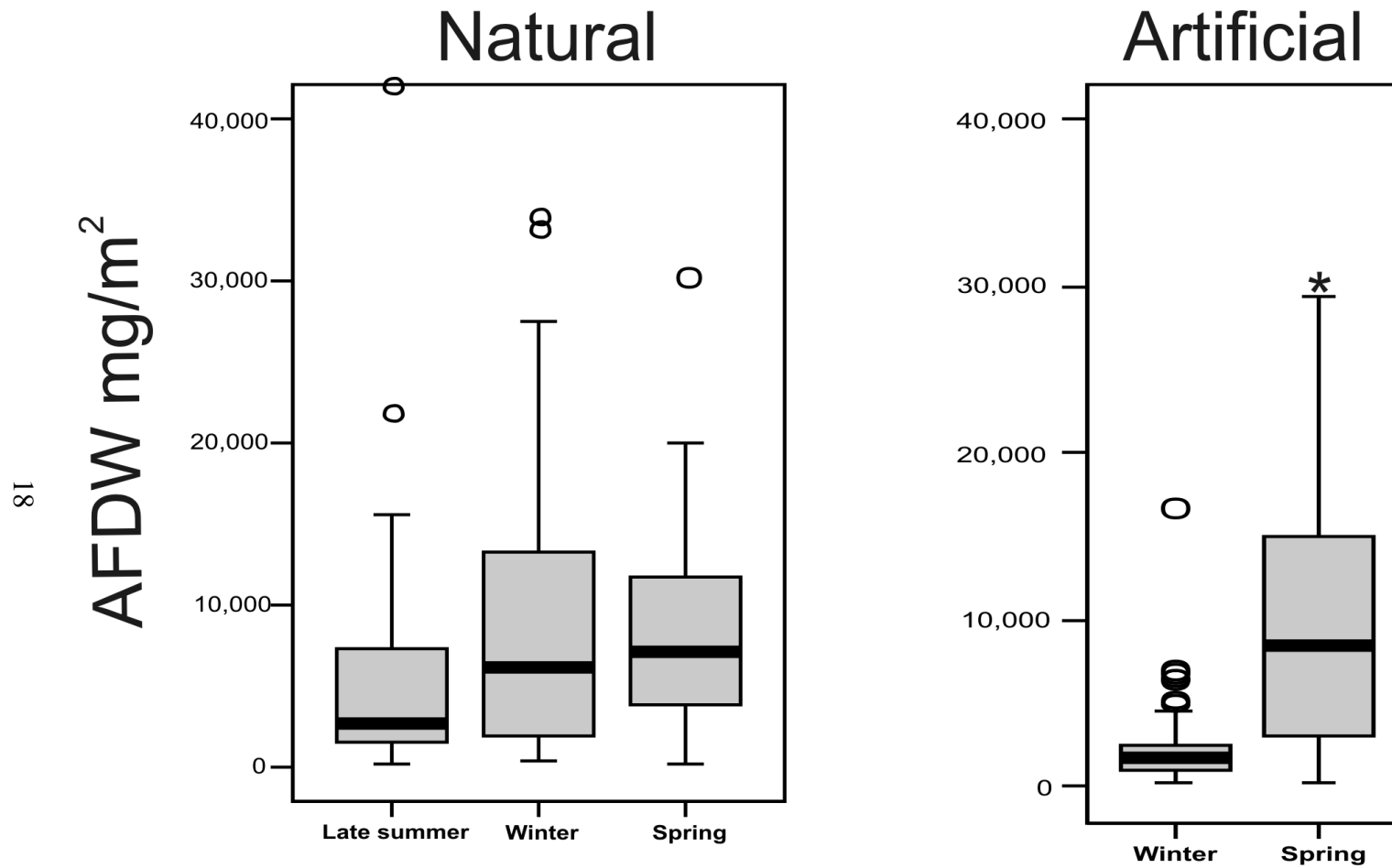
Figure 3. Ash-free dry weight (AFDW) for each season and substrate. AFDW on the natural substrates increased from late summer to winter, and then decreased slightly but not significantly from winter to spring. AFDW on artificial substrates increased significantly from the winter to the spring. (Natural substrates: One Way-ANOVA with Tukey post-hoc test. Artificial substrates: t-test. Asterisks denote seasons which were significantly different from each other. Boxplots: Box height= interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers

Table 2 One-way ANOVA comparison with Tukey post-hoc of seasonal productivity measurements from natural substrates and an independent samples t-test of productivity measurements from artificial substrates. Natural sample sizes were 48 for samples from the winter and late summer and 45 for artificial samples from the spring. Artificial sample sizes were 121 for the winter and 130 for the summer.

Natural Substrates	Late Summer	Winter	Spring	Df	F	P
AFDW		2400	7200	8350	2	2.561	0.082
Chl a		10.3	14.0	14.6	2	2.6	0.076
Artificial Substrates				Df	t	P
AFDW	N/A	1760	8560	81	-6.208	0.000
Chl a	N/A	6.9	8.0	80	2.558	0.012
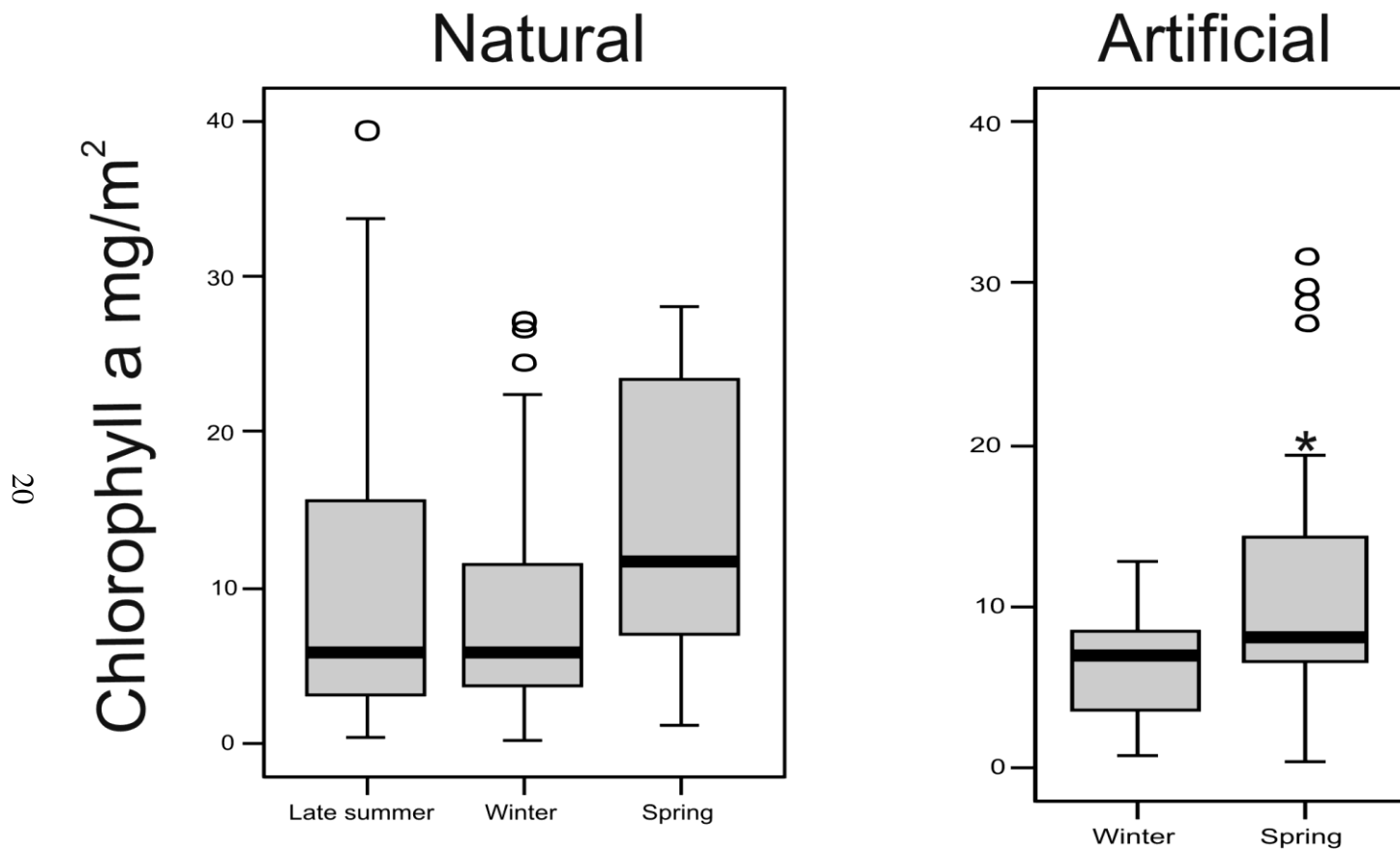
Figure 4. Chlorophyll a for each season and substrate. Chlorophyll a on natural substrates decreased slightly but not significantly from the late summer to the winter, and increased slightly but not significantly in the spring. Chlorophyll a on artificial substrates increased significantly from the winter to the spring. (Natural substrates: One Way-ANOVA with Tukey post-hoc test. Artificial substrates: t-test. Asterisks denotes seasons which were significantly different from each other. Boxplots: Box height= interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers)
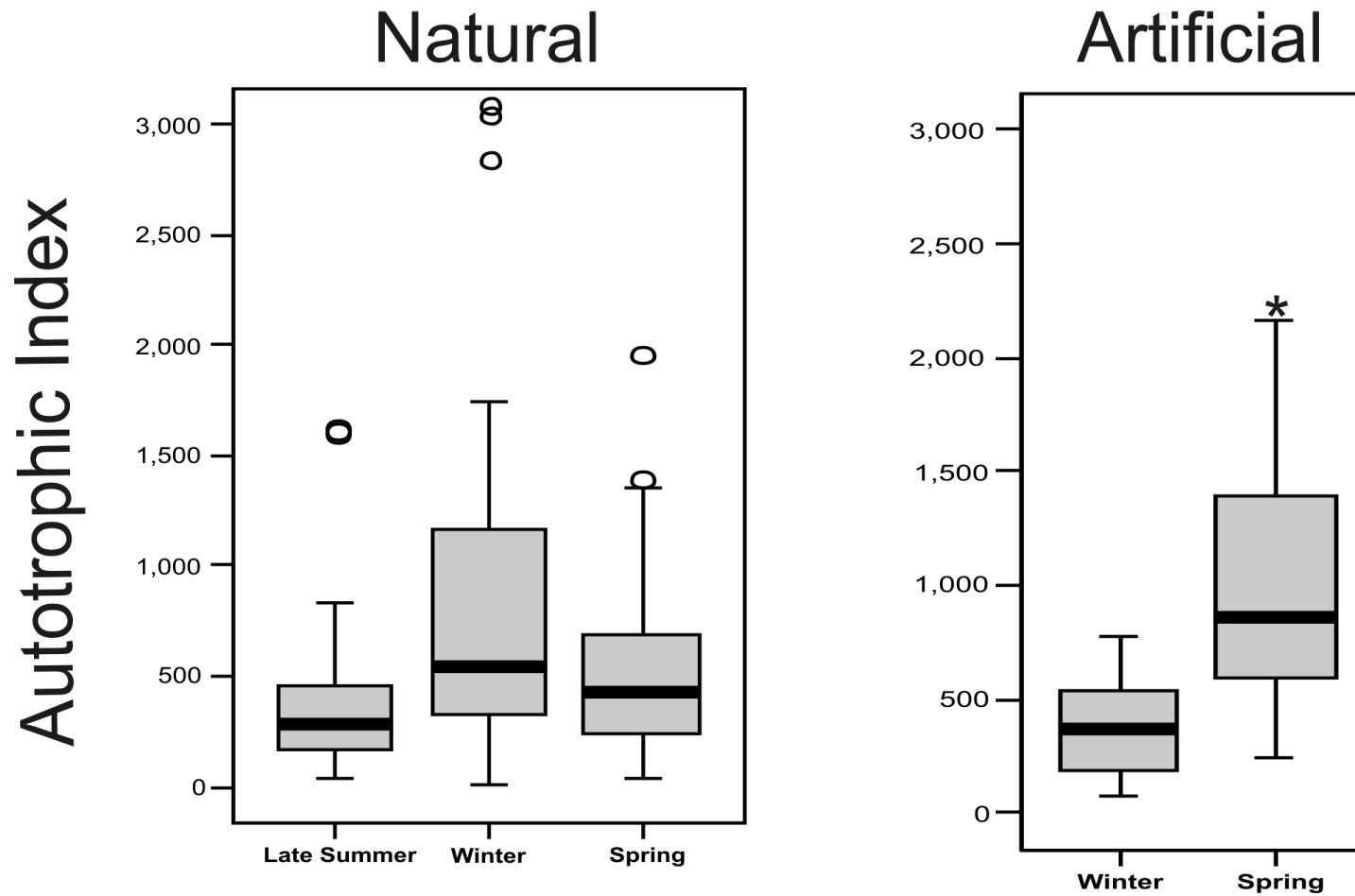
Figure 5. Autotrophic Index (AI) for each season. On the natural substrates the AI increased slightly but not significantly from the late summer to the winter, and decreased slightly, but not significantly, in the spring. On the artificial substrates the AI increased significantly from the winter to the spring. (Natural substrates: One Way-ANOVA with Tukey post-hoc test. Artificial substrates: t-test. Asterisks denote seasons which were significantly different from each other. Boxplots: Box height= interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers)
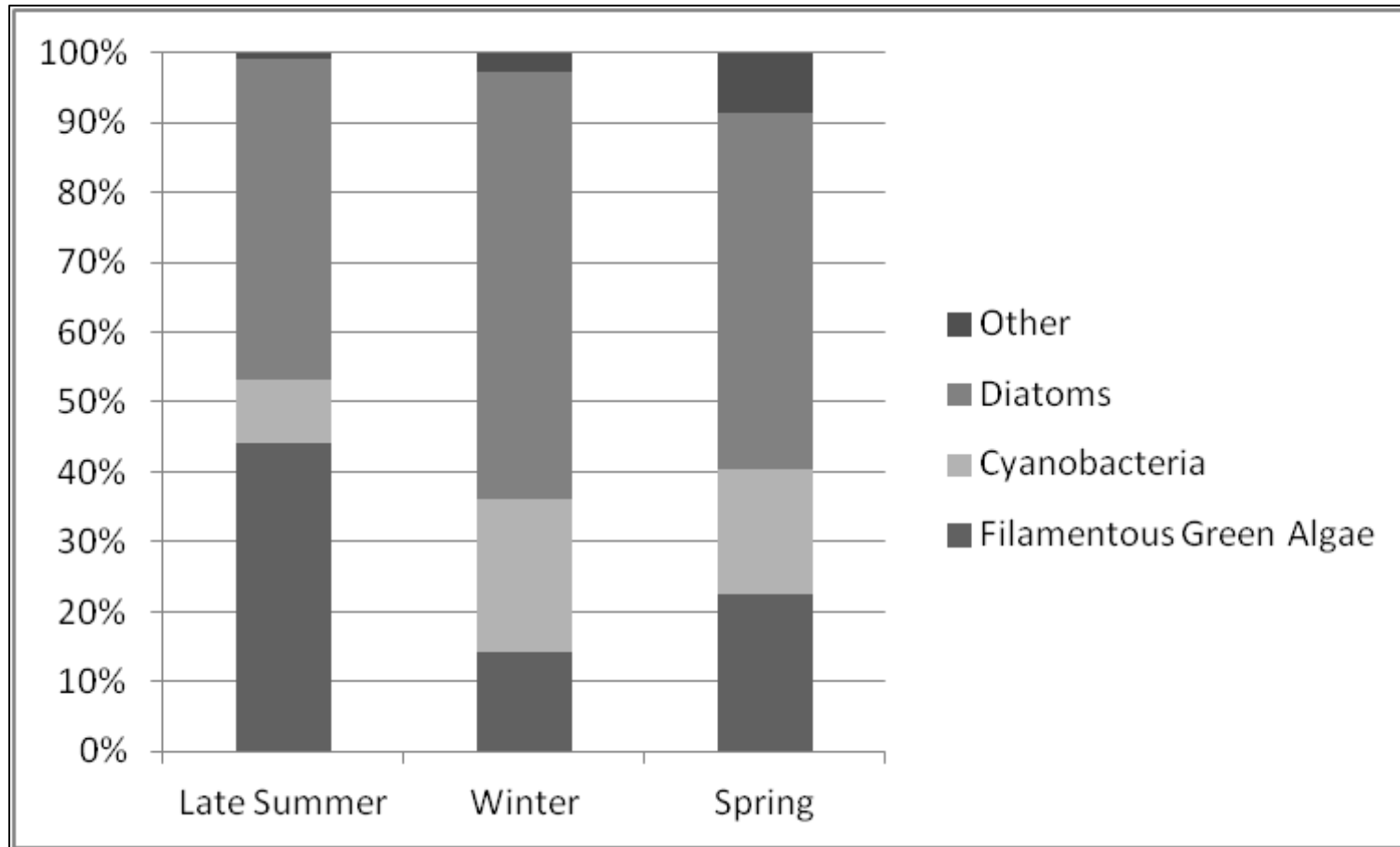
Figure 6. Periphyton community composition from all substrates by season. Filamentous green algae were at lowest abundance in the winter but increased in abundance in spring and late summer. Diatoms were always abundant but peaked in the winter. Cyanobacteria were usually less prevalent than were filamentous green algae and diatoms. Cyanobacteria abundance was lowest in the late summer, increasing in the winter, and decreasing again in the spring. Other algae made up only a small percentage of the total cell counts during each season, but were lowest in the late summer and highest in the spring

Differences among Sites in Productivity and Organic Material

Sites with a gradual offshore slope almost always tended to have higher AFDW, although this trend was only significant on natural substrates during the winter (Figure 7, a summary of the results from all GLM tests can be found in Table 3 and full data in the appendix). Sites with a gradual offshore slope also had significantly higher chlorophyll a in several cases which was representative of the general trend except on artificial substrates during the winter in which sites with gradual offshore slopes had less chlorophyll a (Figure 8). Sites with low conifer cover tended to have higher AFDW and chlorophyll a, although this was only significant for AFDW on natural substrates during the winter (Figures 9-10). Sites with high alder cover had significantly higher AFDW and chlorophyll a in one case each, which were generally representative of the trends except during the spring in which sites with high alder cover had significantly lower AFDW (Figures 11-12). Development seemed to have had little effect on AFDW and chlorophyll a for which there were no significant differences or obvious trends (Figures 13-14).

The autotrophic index, AI, is an index of the proportion of organic biomass which is likely to be active in photosynthesis as indicated by the presence of chlorophyll a. Counter-intuitively, a high numerical AI is associated with a low proportion of chlorophyll. The general AI trend in this study was toward a higher AI on gradual slopes than on steep slopes. However, this relationship was significant only on artificial substrates during the winter (Figure 15). A comparison of the AFDW and chlorophyll a relationships that go into producing the AI indicates that this trend was mainly due to
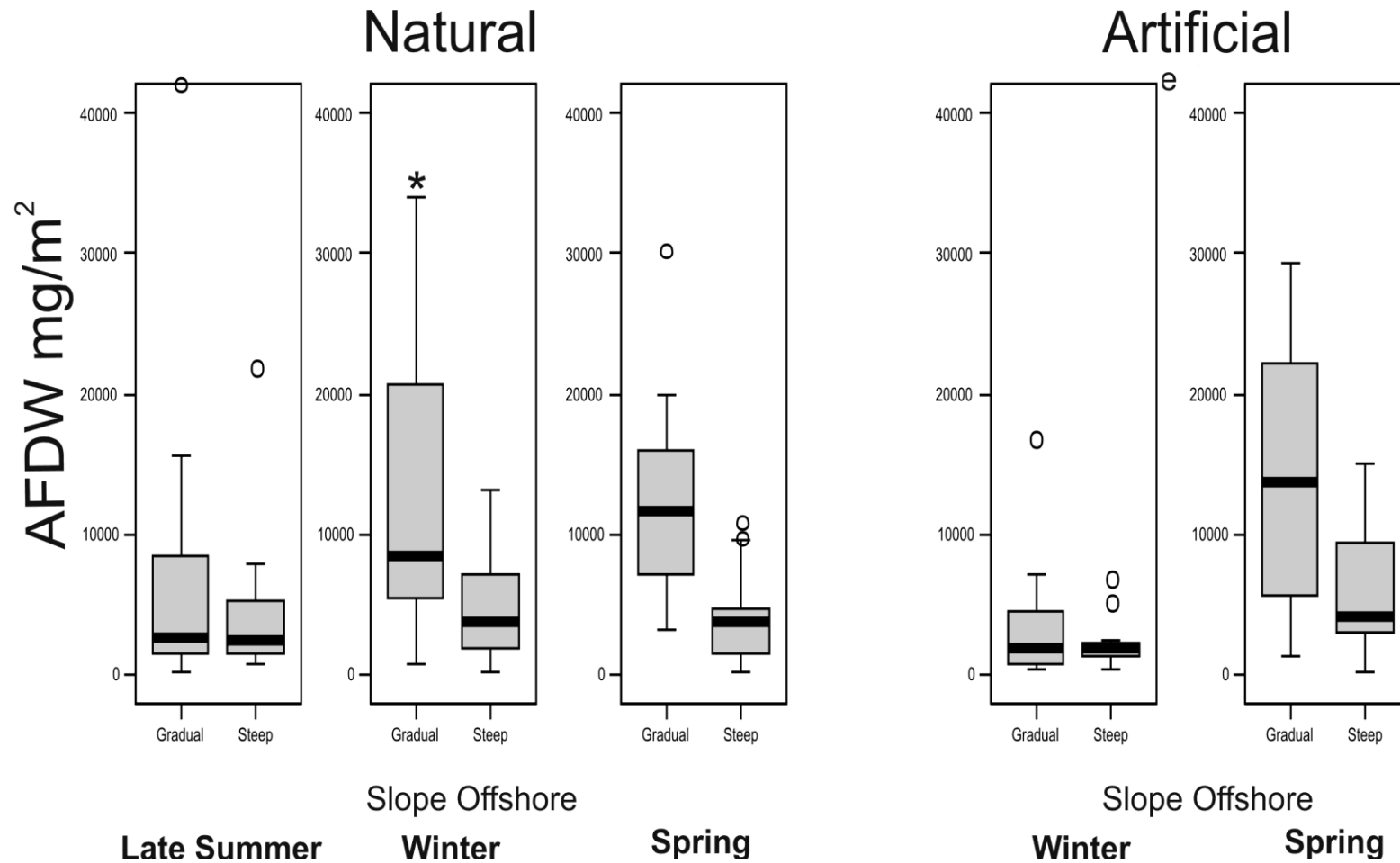
Figure 7. AFDW as a function of the slope offshore. AFDW trended higher at sites with a gradual offshore slope for all seasons and substrates, but was significantly higher only during the winter on natural substrates (General Linear Model. Asterisks denote significant differences. Boxplots Box height= interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers)

**Table 3.** Summary of GLM results. Groups that showed a significant difference are labelled with a letter. If sites with a gradual slope had significantly higher results then a "G" is given, and if sites with steep slopes had significantly higher results then an "S" is given. If sites with low tree cover had significantly higher results then an "L" is given, and if sites with high tree cover had significantly higher results then an "H" is given. If undeveloped sites had significantly higher results then a "U" is given, and if developed sites had significantly higher results then a "D" is given. Differences are significant at the 0.05 level.

		Natural			Artificial		
		Late Summer	Winter	Spring	Winter	Spring	Recount
Offshore Slope							
	AFDW		G		G		
	Chlorophyll a		G	G			
	AI				G		
Conifer Cover							
	AFDW		L				
	Chlorophyll a						
	AI						
Alder Cover							
	AFDW		H			L	
	Chlorophyll a		H				
	AI					L	
Development							
	AFDW						
	Chlorophyll a						
	AI		D		D		
Offshore Slope							
	Filamentous	G	G			G	G
	Cyanobacteria					G	
	Diatoms	S	S		G	S	S
	Other cells						
Conifer Cover							
	Filamentous						
	Cyanobacteria						
	Diatoms						
	Other cells		L		L		
Alder Cover							
	Filamentous	H	H			H	
	Cyanobacteria					H	H
	Diatoms	L			L	L	L
	Other cells				H		
Development							
	Filamentous						
	Cyanobacteria			D			
	Diatoms						
	Other cells						
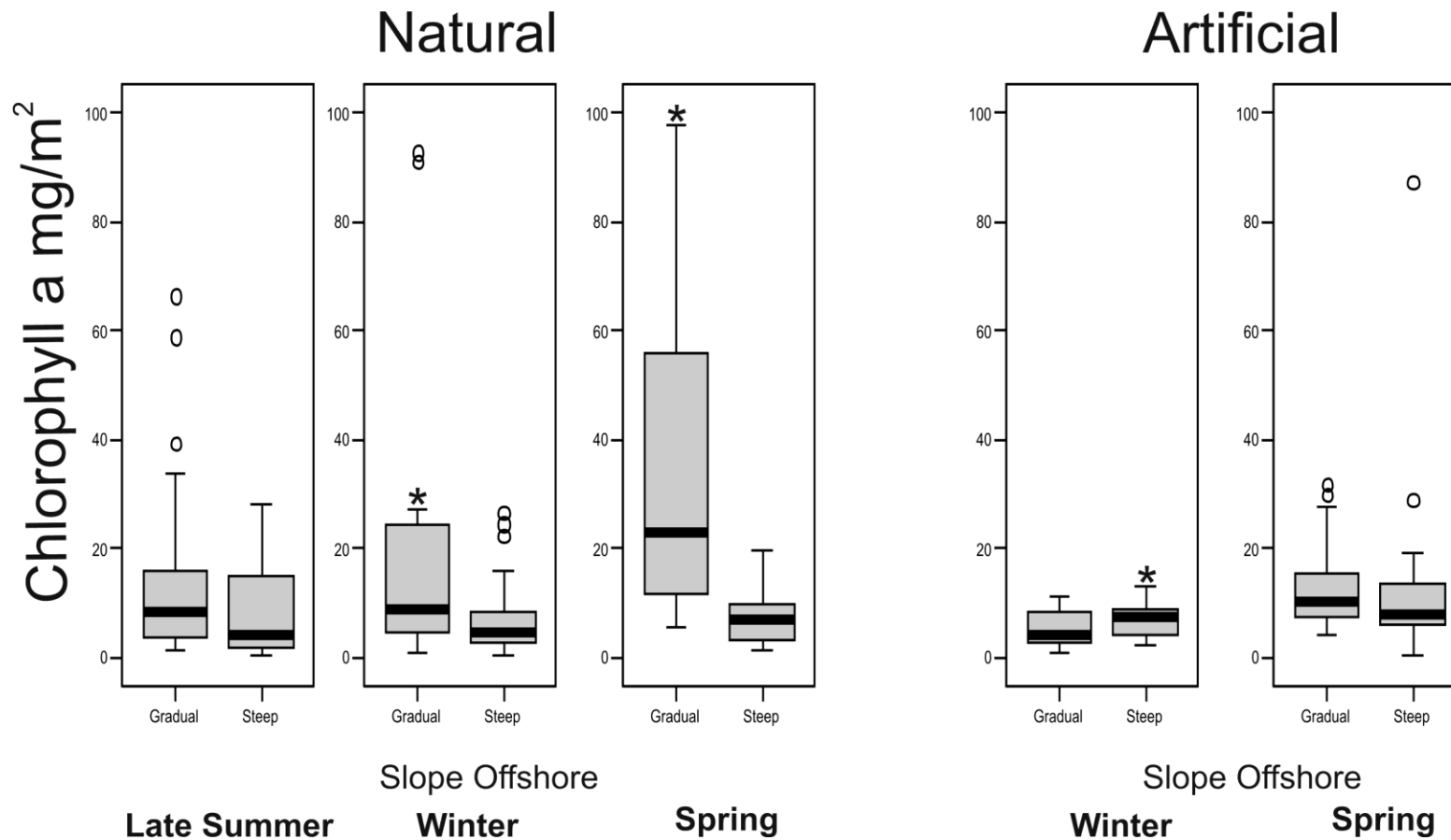
Figure 8. Chlorophyll a as a function of the slope offshore. Chlorophyll a tended to be higher and in several instances was significantly higher on substrates from shorelines with a gradual offshore slope than from shorelines with a steep slope. Artificial substrates from gradual slopes in the winter, however, had chlorophyll a levels significantly lower than those from steep slopes (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band= median. Error bars=range excluding outliers. O=outliers).
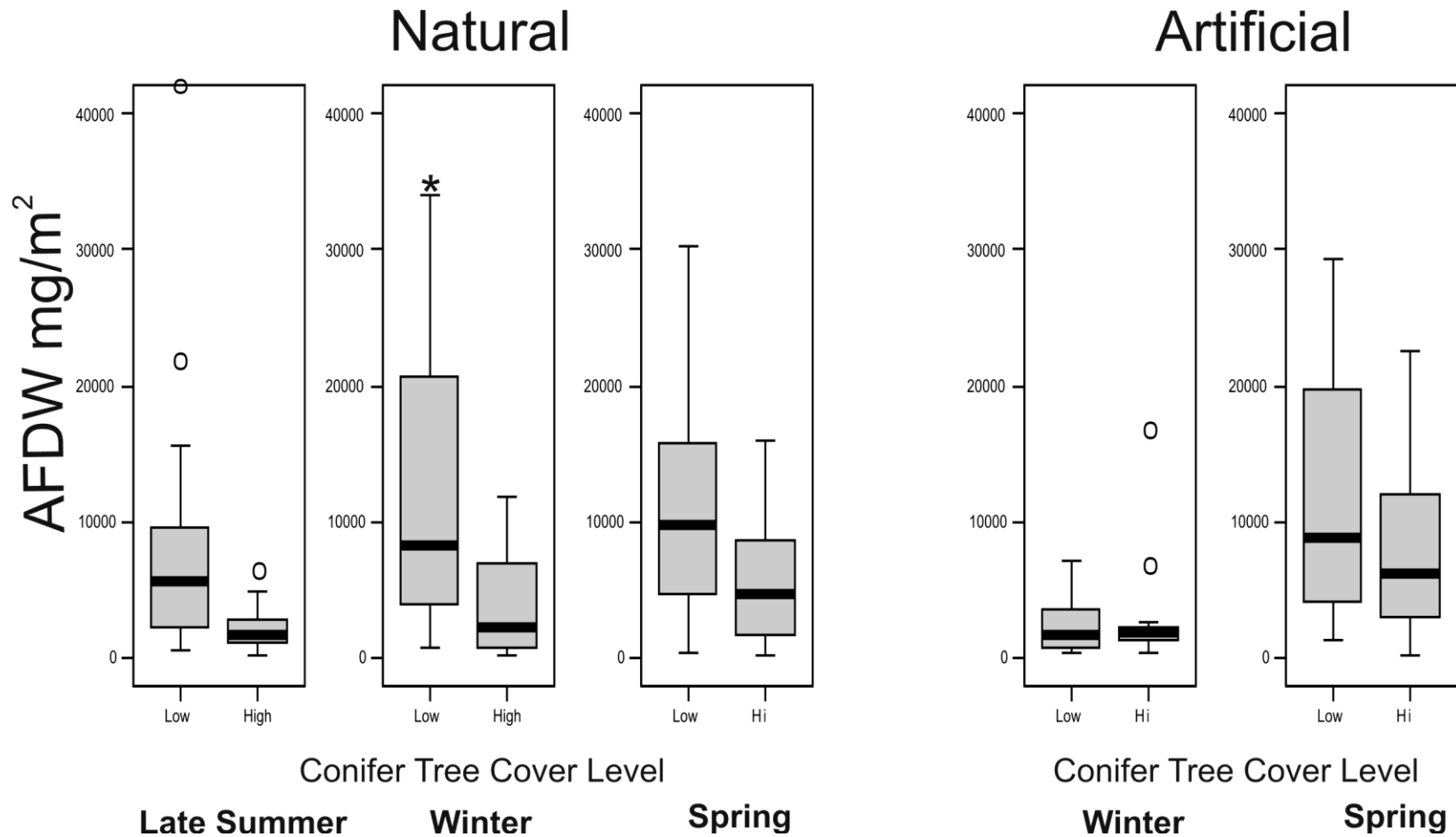
Figure 9. AFDW as a function of conifer tree cover. AFDW trended higher at sites with low conifer cover for all seasons and substrates, but was only significantly higher on natural substrates during winter (General Linear Model. Asterisks denote significant differences. Box plots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers.)
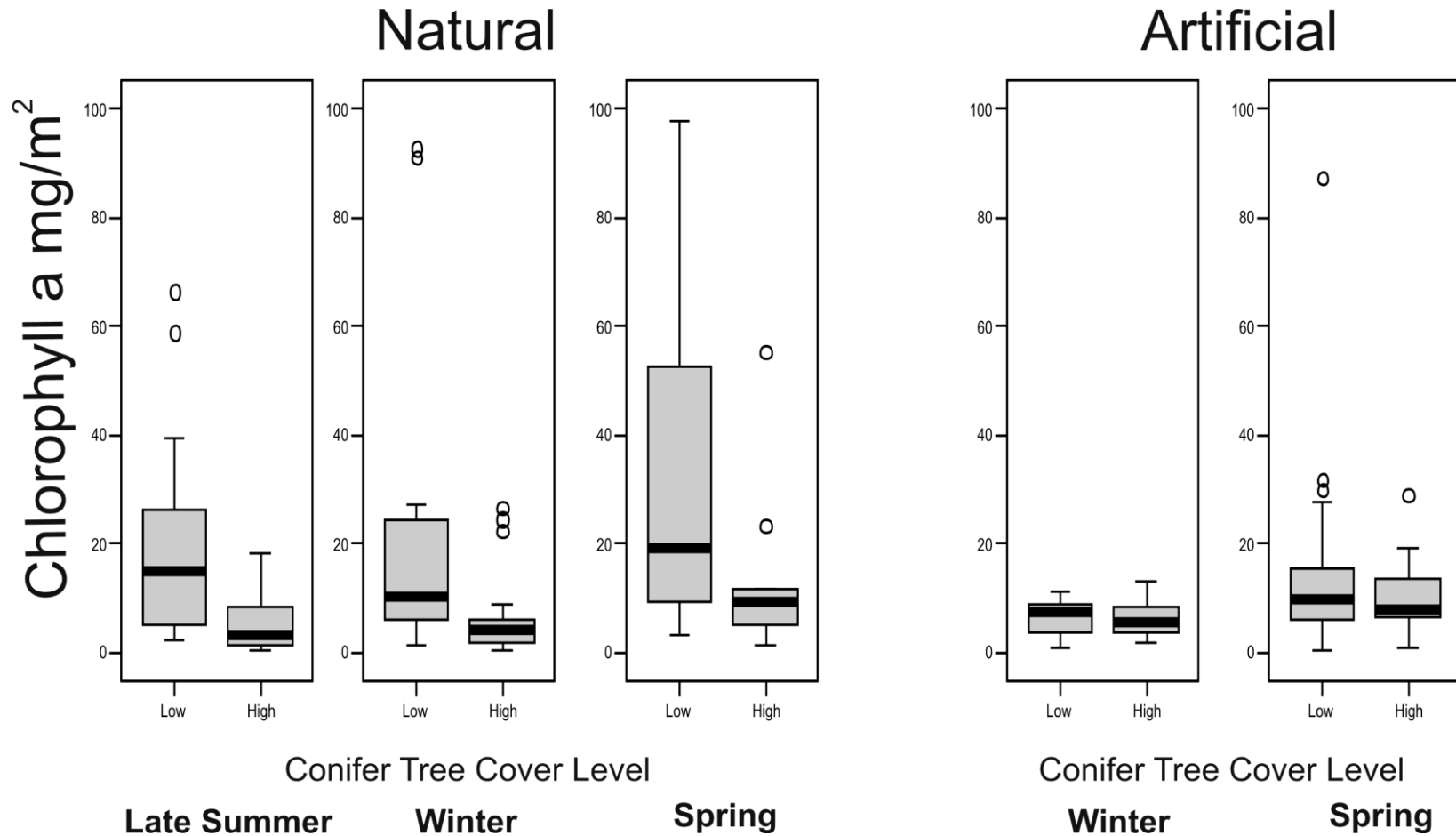
Figure 10. Chlorophyll a as a function of conifer tree cover. No significant differences were seen at any season (General Linear Model). The general trend was toward lower chlorophyll a at sites with higher conifer cover. (Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
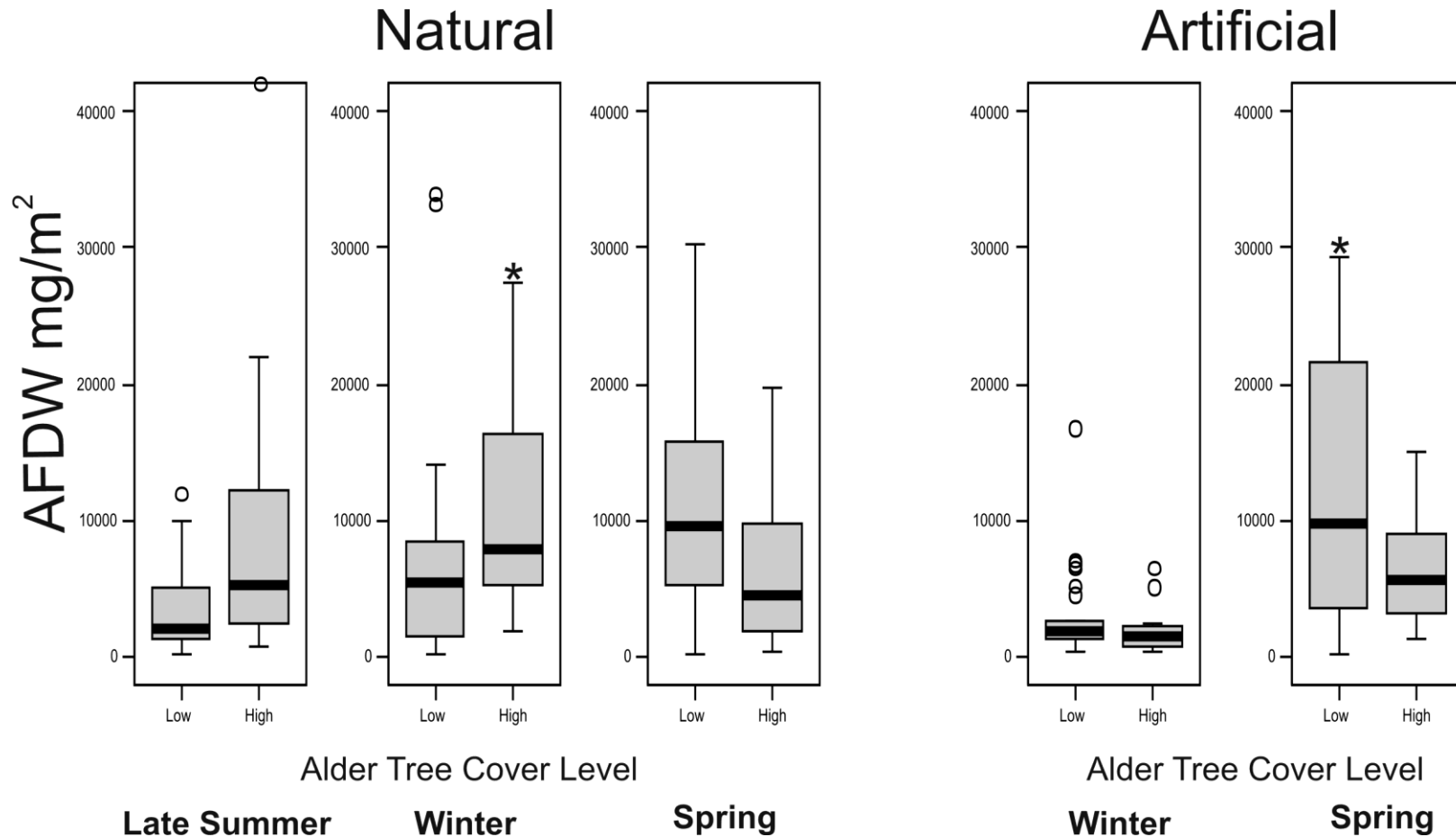
Figure 11. AFDW as a function of alder tree cover. AFDW trended higher at sites with high alder cover on natural substrates during the fall and winter, but lower during the spring on natural substrates and during the winter and spring on artificial substrates. The two significant differences were on natural substrates during winter and artificial substrates during the spring (General Linear Model Asterisks denote significant differences. Boxplots; Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
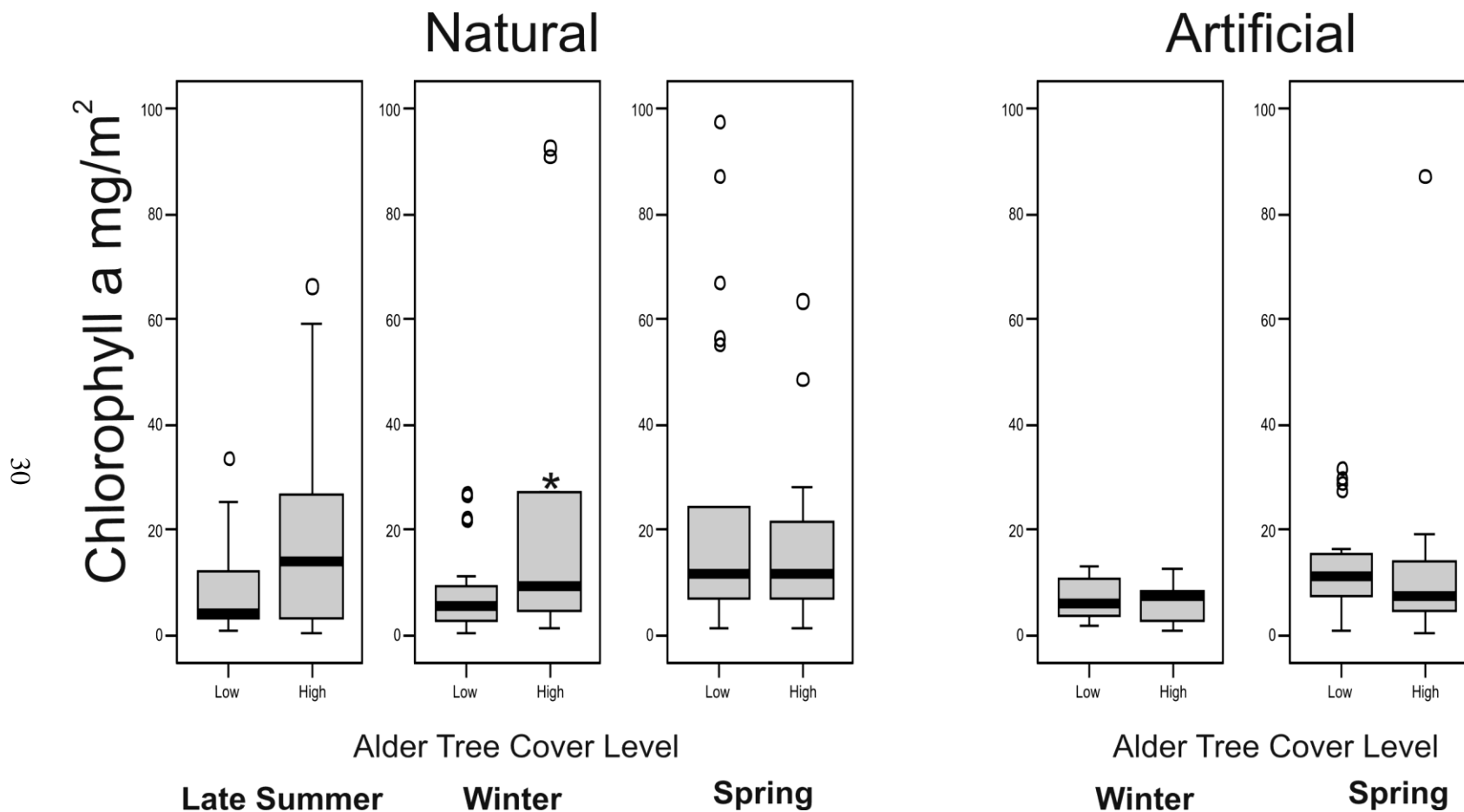
Figure 12. Chlorophyll a as a function of alder tree level. Chlorophyll a tended to be higher from sites with high alder cover during all seasons except for artificial substrates during the spring. This difference was only significant during the winter on natural substrates (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
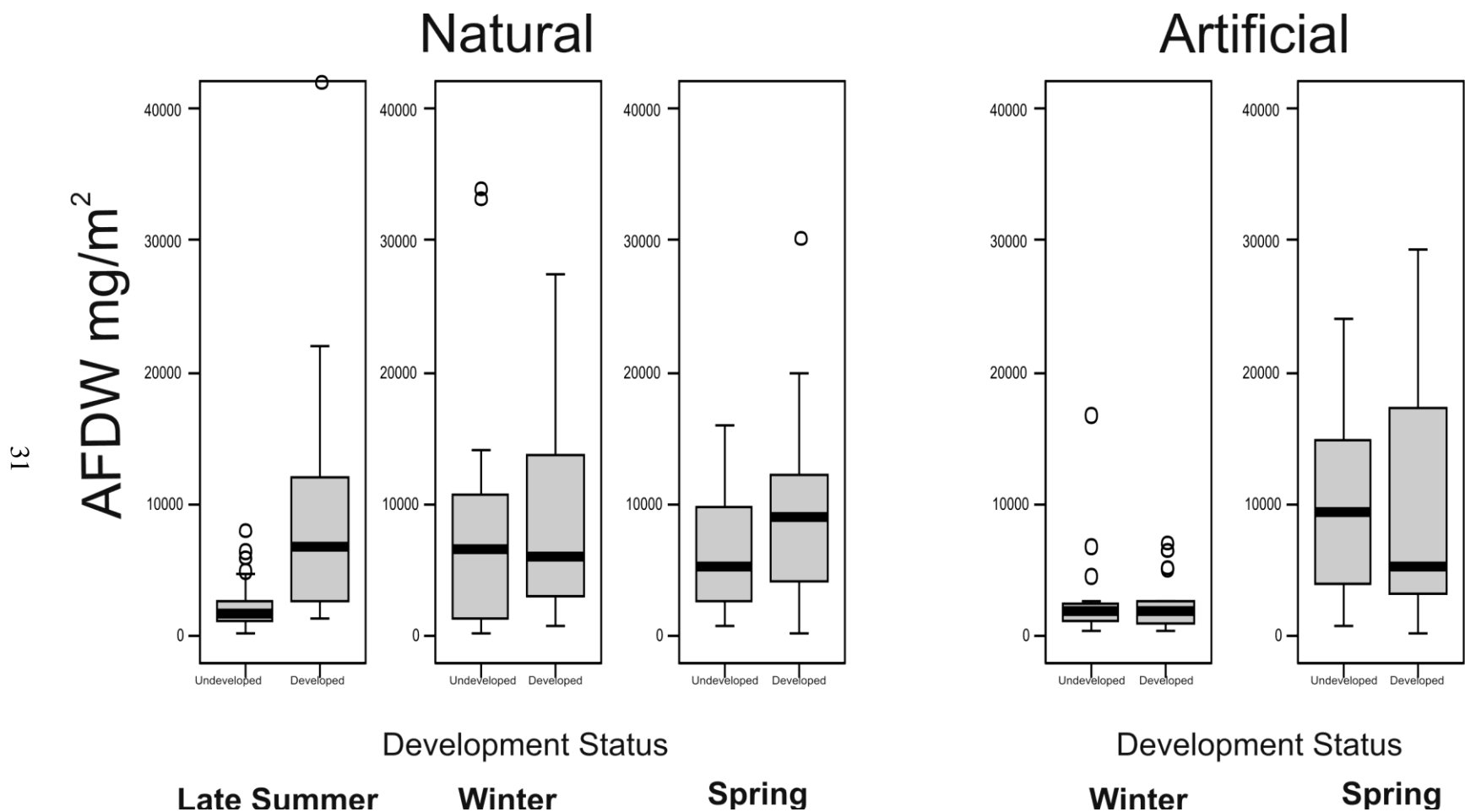
Figure 13. AFDW as a function of development status. There were no significant differences in AFDW by development status at any season, though a possible trend was toward higher AFDW at developed sites on natural substrates but lower on artificial substrates (General Linear Model. Asterisks denote significant differences. Boxplots: Box height= interquartile range. Central band=median. Error bars= range excluding outliers. O=outliers)

Figure 14. Chlorophyll a as a function of development status. No significant differences in chlorophyll a levels were found between developed and undeveloped sites during any season (General Linear Model). The general trend, however, was toward slightly higher chlorophyll a levels at developed sites. (Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
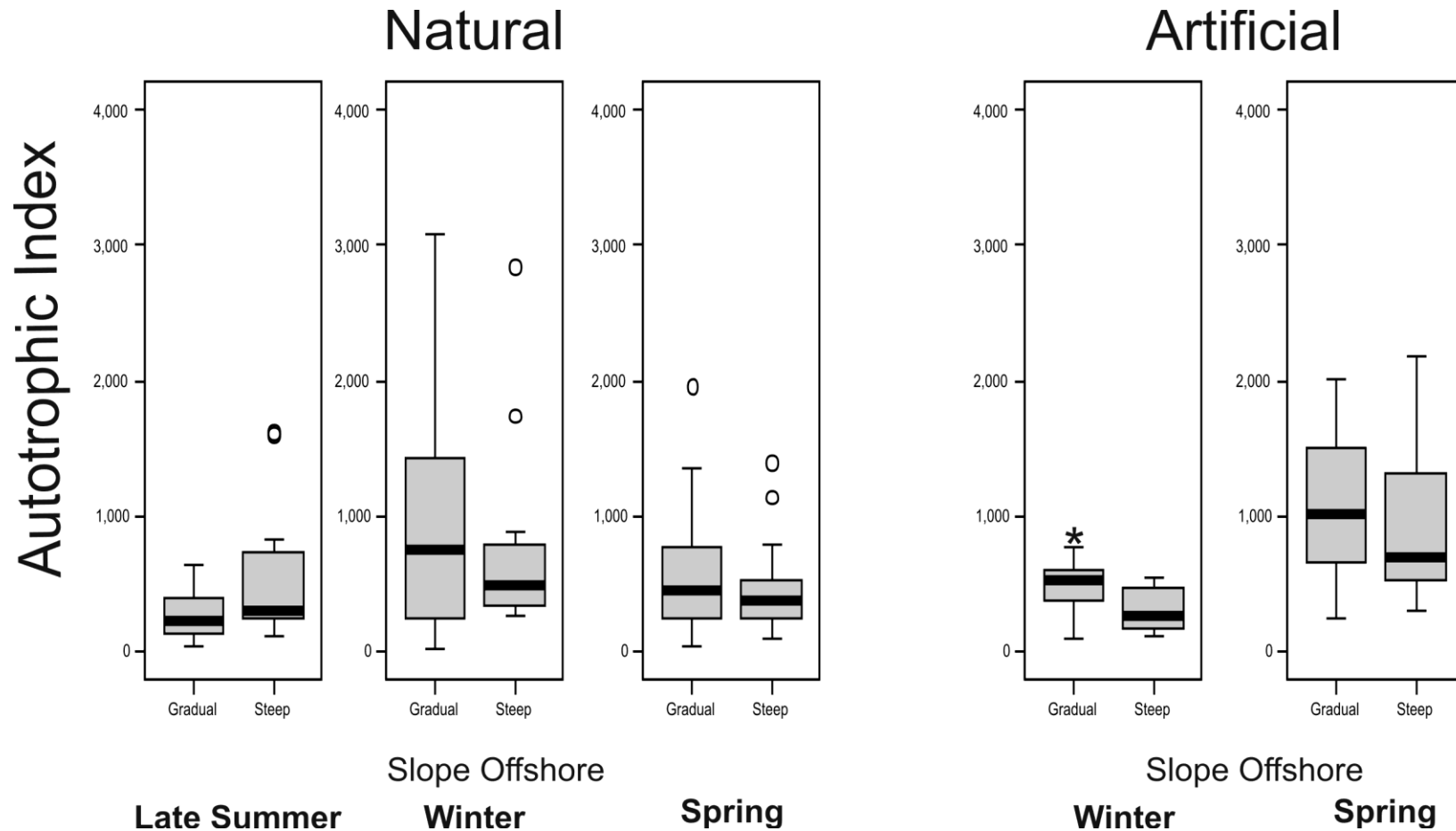
Figure 15. Autotrophic index (AI) as a function of slope offshore. A high autotrophic index indicates a low chlorophyll a to organic material ratio. The general trend was toward a lower AI on steep slopes than on gradual slopes. This relationship was significant on artificial substrates during the winter (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers). Comparison of AFDW and chlorophyll a relationships indicates that this trend was mainly due to lower AFDW on steep slope

lower AFDW on steep slopes (Figure 7). AI had no significant correlation with conifer or alder cover or with development status (Figures 16-18).

Periphyton Algal Community

Sites with gradual offshore slopes tended to have a significantly higher proportion of filamentous green algae in several cases, especially in late summer and winter (Figure 19). Offshore slope had little consistent effect on the proportion of cyanobacteria or other algae, except for a few occasions in which gradual slopes had higher levels of these groups. (Figures 20-21). Diatoms, in contrast, usually tended to be more prevalent on shores with steep offshore gradients, a trend which was often significant (Figure 22).

Conifer cover seemed to have little or no significant effect on periphyton community composition except other algae which was significantly higher at sites with low conifer cover on both natural and artificial substrates during the winter (Figures 23-26). Diatoms also usually tended to be a larger part of the algal community on sites with high conifer cover, though this trend was not significant (Figure 25).

Sites with high alder cover usually had higher proportions of filamentous green algae, a trend which was significant in several cases (Figure 27). Cyanobacteria also usually tended to be slightly more abundant in areas with high alder cover, though this trend was significant only in spring on artificial substrates (Figure 28). In contrast, diatoms were consistently less abundant at sites with high alder cover, significantly so in several instances (Figure 29). Alder cover seemed to have very little consistent effect on other algae (Figure 30).
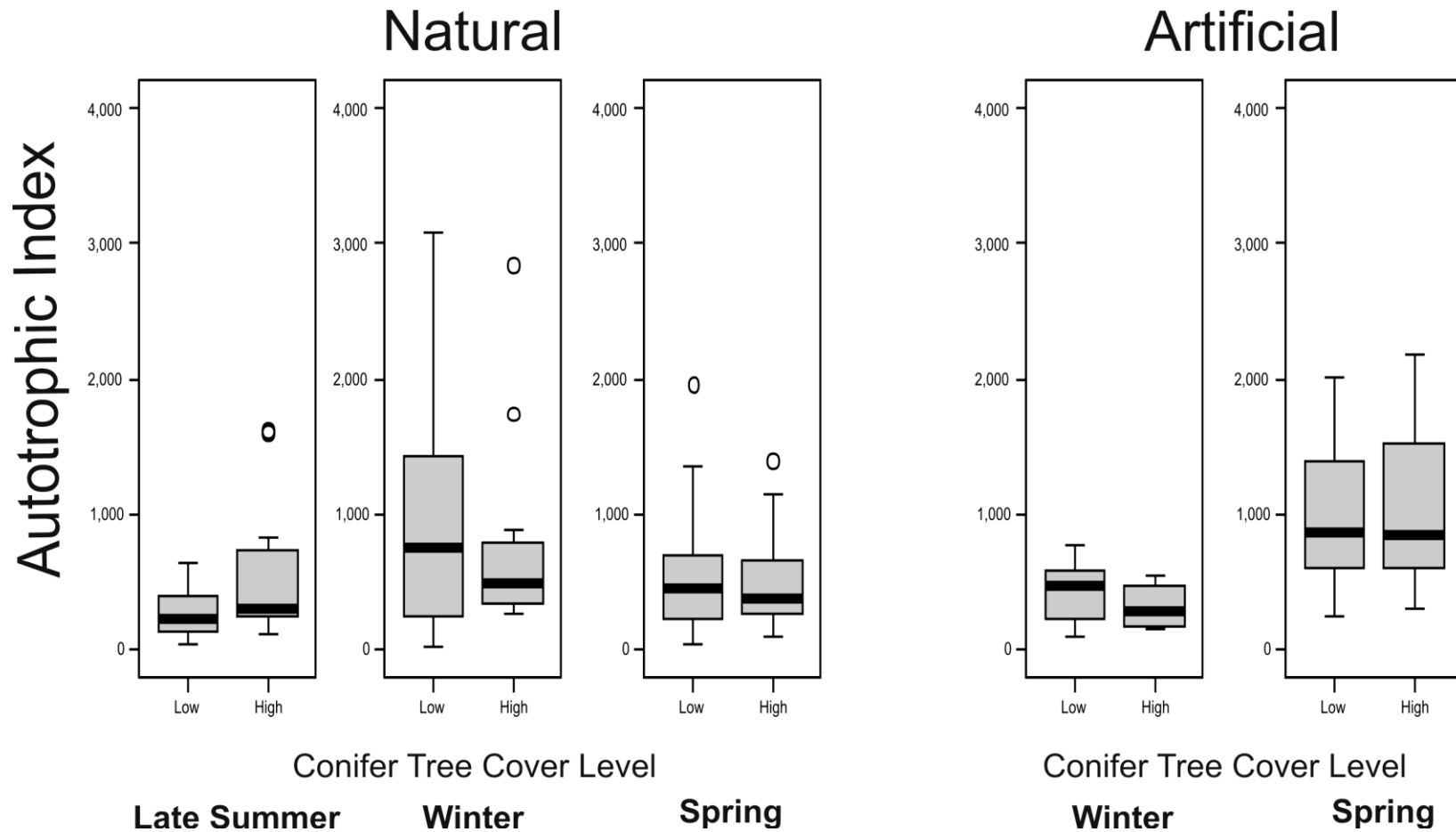
Figure 16. Autotrophic index (AI) as a function of conifer tree cover. A high AI indicates a low chlorophyll a to organic material ratio. Sites with low conifer cover tended to have a higher AI except on natural substrates during the late summer. No differences in AI were significant (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars= range excluding outliers. O=outliers).
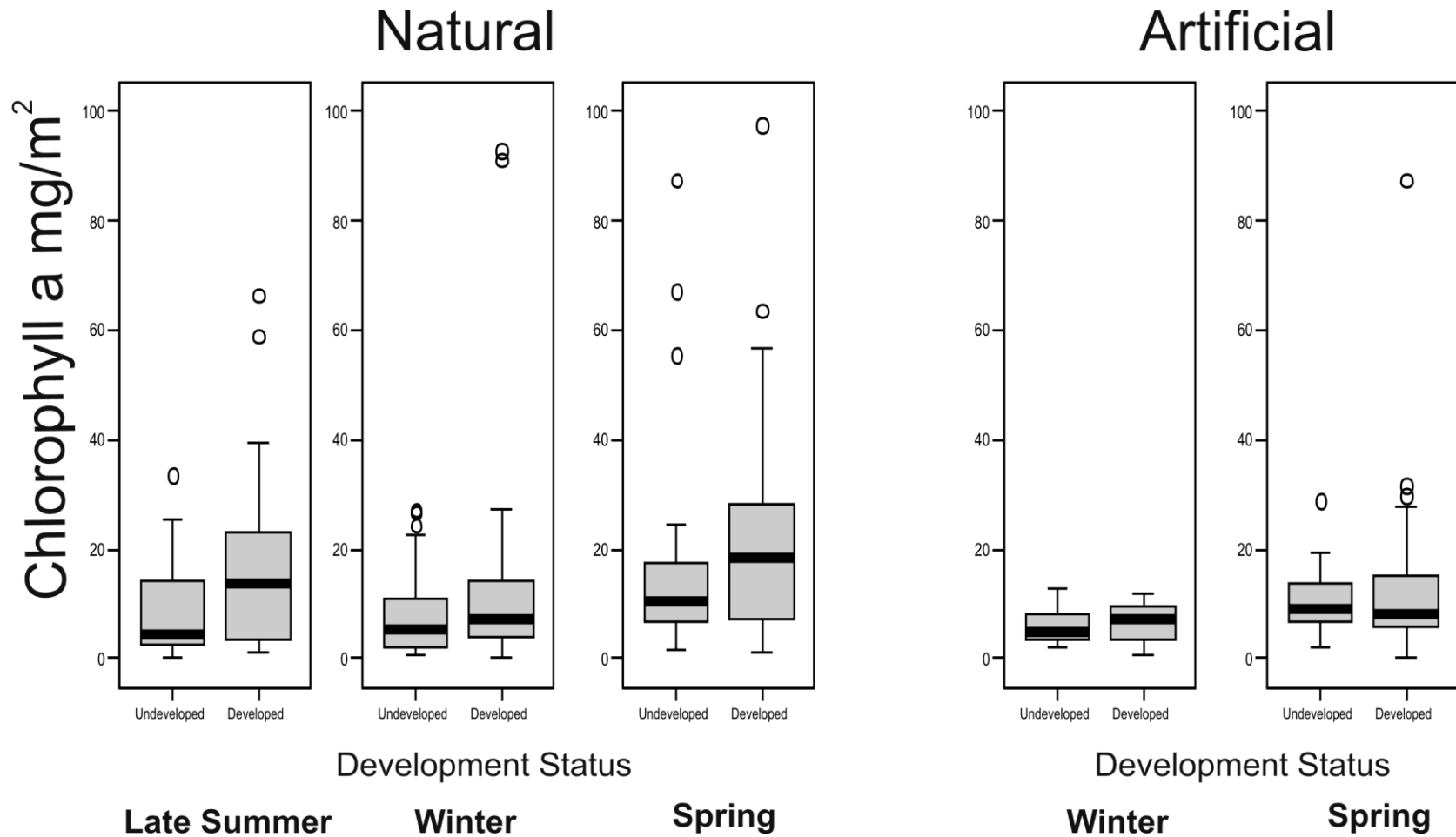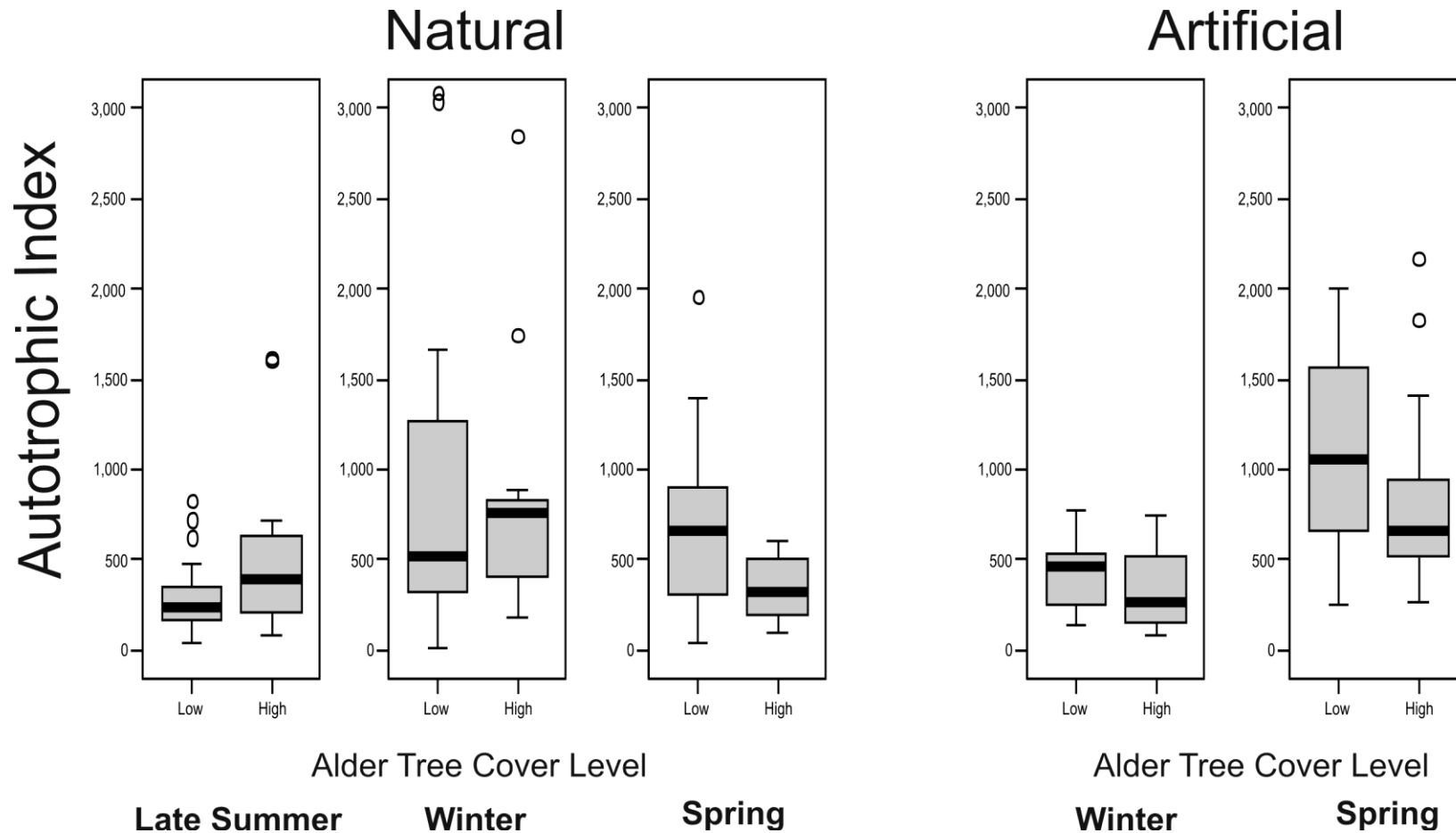
Figure 17. Autotrophic index (AI) as a function of alder tree cover. A high AI indicates a low chlorophyll a to organic material ratio. There were no significant relationships between alder tree cover and AI (General Linear Model). The trend was toward higher AI (lower chlorophyll a/organic material) on natural substrates with high alder cover in summer and winter, but toward lower AI at sites with high alder cover in the spring and on all artificial substrates (Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outlier).
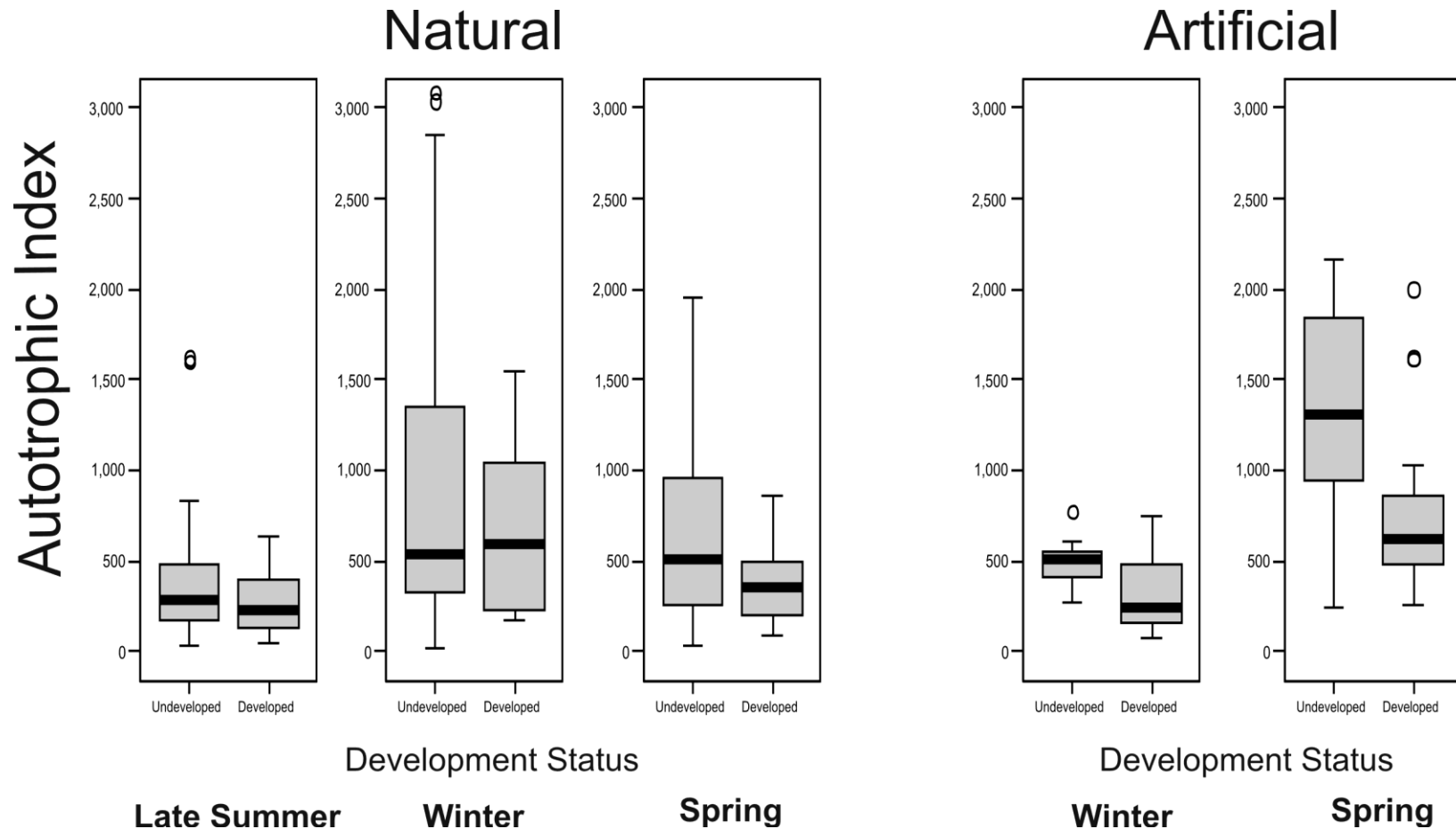
Figure 18. Autotrophic index (AI) as a function of development status. There were no significant differences in AI between developed and undeveloped sites (General Linear Model). The general trend, however, was toward lower AI (more chlorophyll a/organic material) at developed sites. (Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).

Figure 19. Proportion of filamentous green algae as a function of slope offshore. More filamentous green algae were found on all substrates from sites with a gradual slope during all seasons except for natural substrates during spring. Filamentous green algae counts were significantly different on natural substrates during the late summer, artificial substrates during the spring, and natural substrates during the winter (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
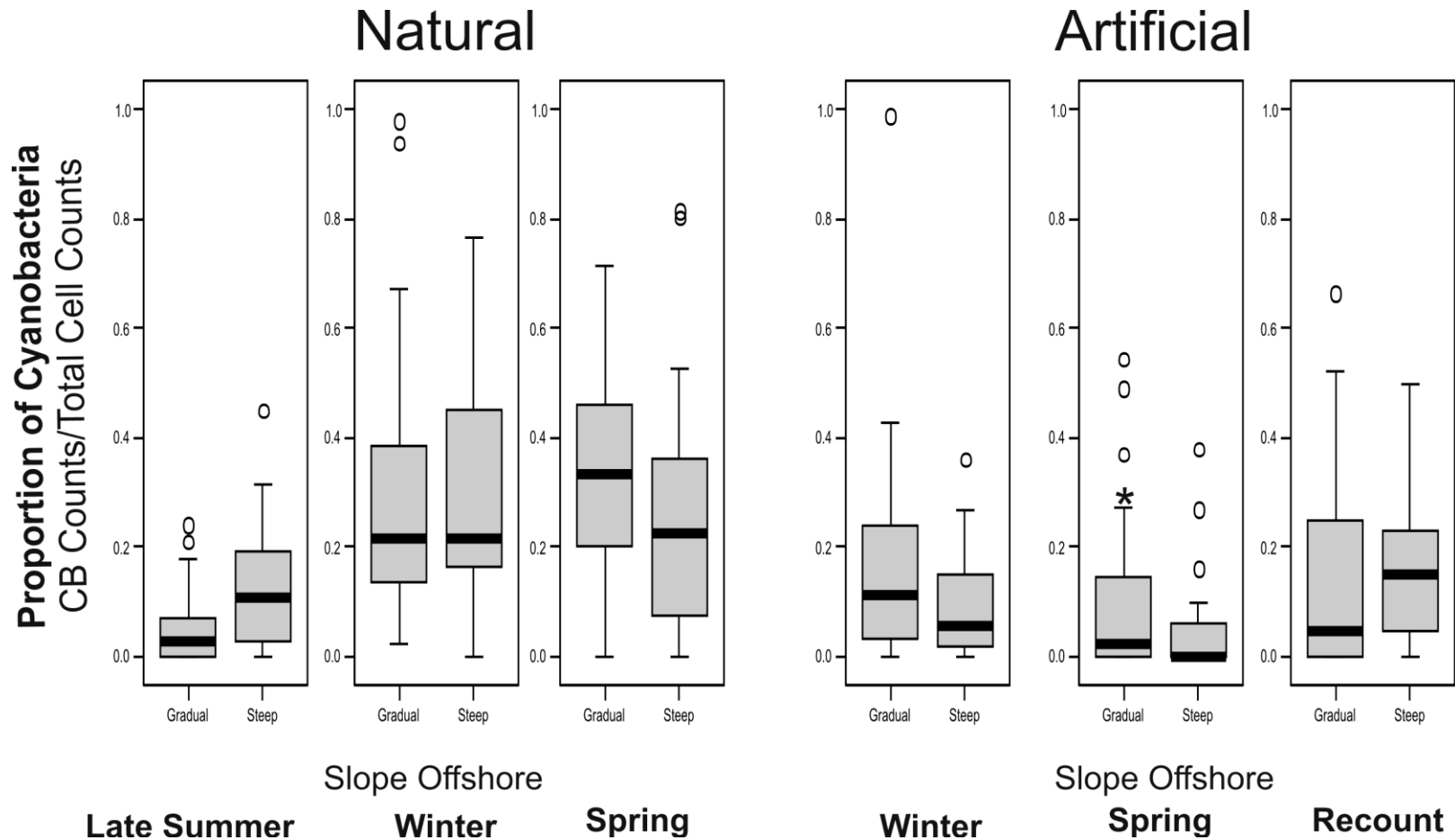
Figure 20. Proportion of cyanobacteria (CB) as a function of the slope offshore. Sites with a gradual slope off shore generally had more abundance of cyanobacteria except for the spring recounts from artificial substrates. Cyanobacteria counts were significantly different on natural substrates during the late summer and on artificial substrates in the spring (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
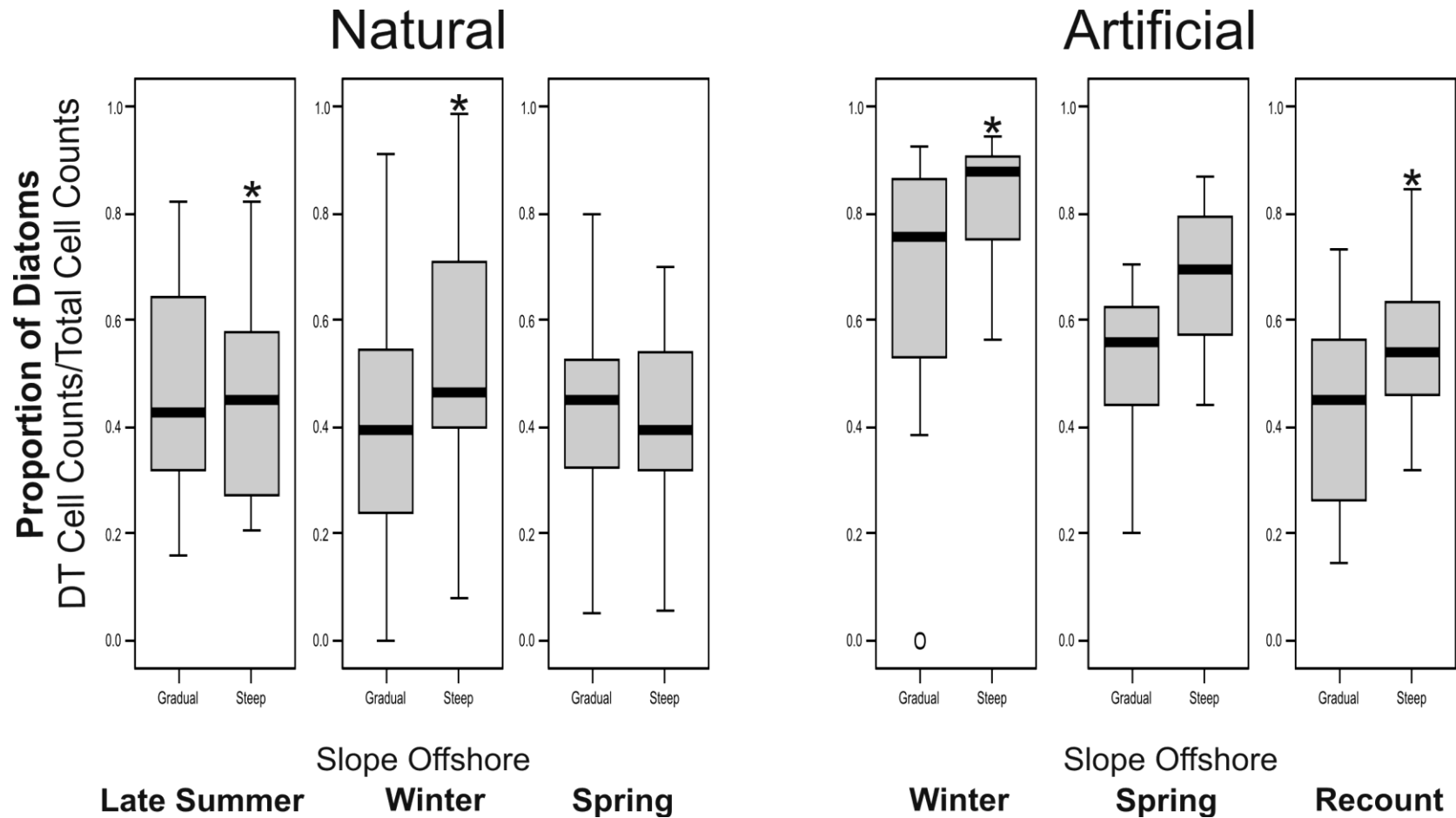
Figure 21. Proportion of diatoms (DT) as a function of slope offshore. DT abundance tended to be higher at sites with steep slopes, significantly so during the winter on both substrates, and on the spring artificial recounts. However, DT abundance was significantly higher at sites with gradual offshore slopes during the late summer on natural substrates (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
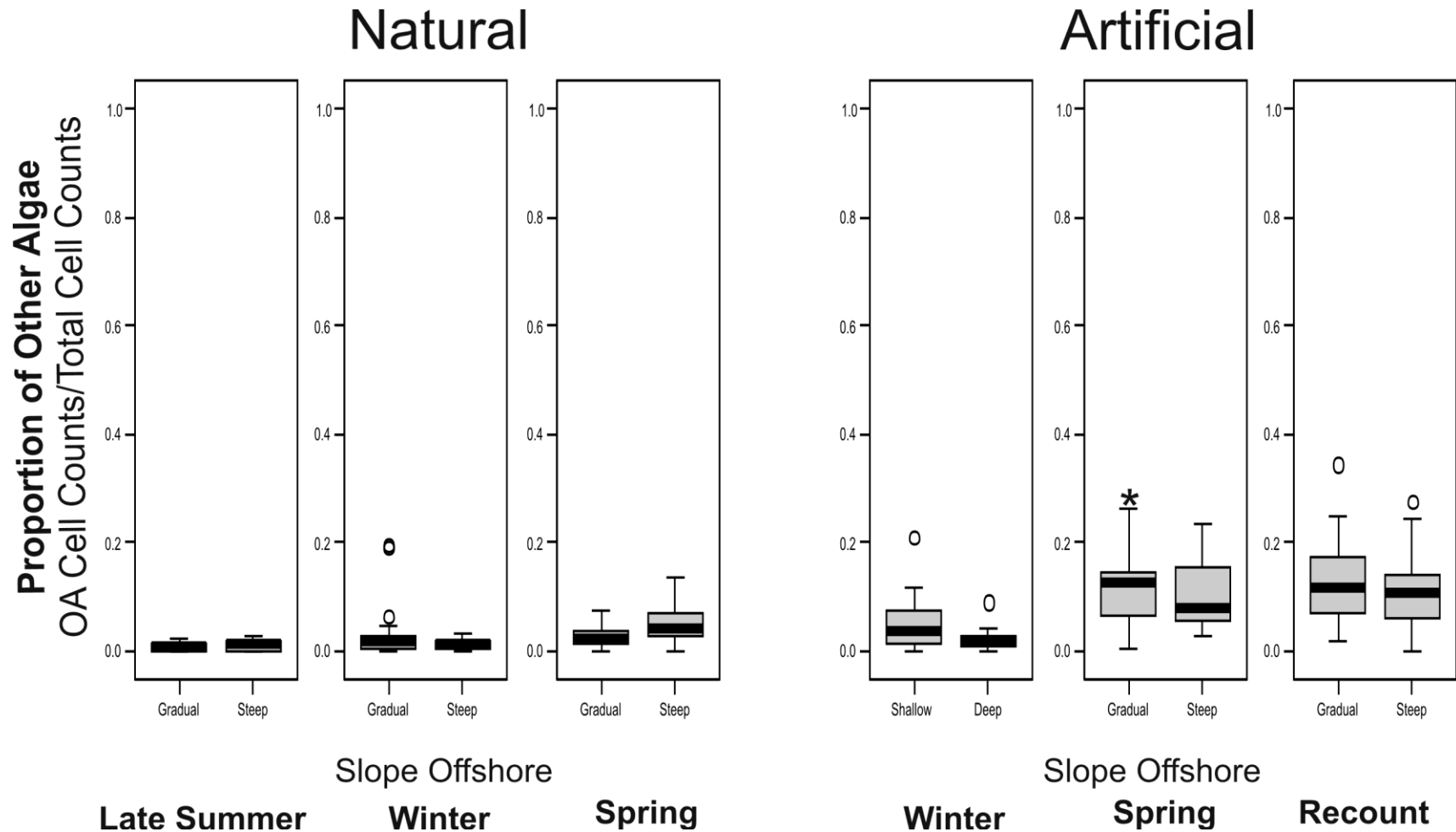
Figure 22. Proportion of other algae (OA) as a function of slope offshore. OA abundance tended to be very similar between sites with gradual and steep slopes offshore, except on artificial substrates during the spring in which OA were significantly higher at sites with a gradual offshore slope (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
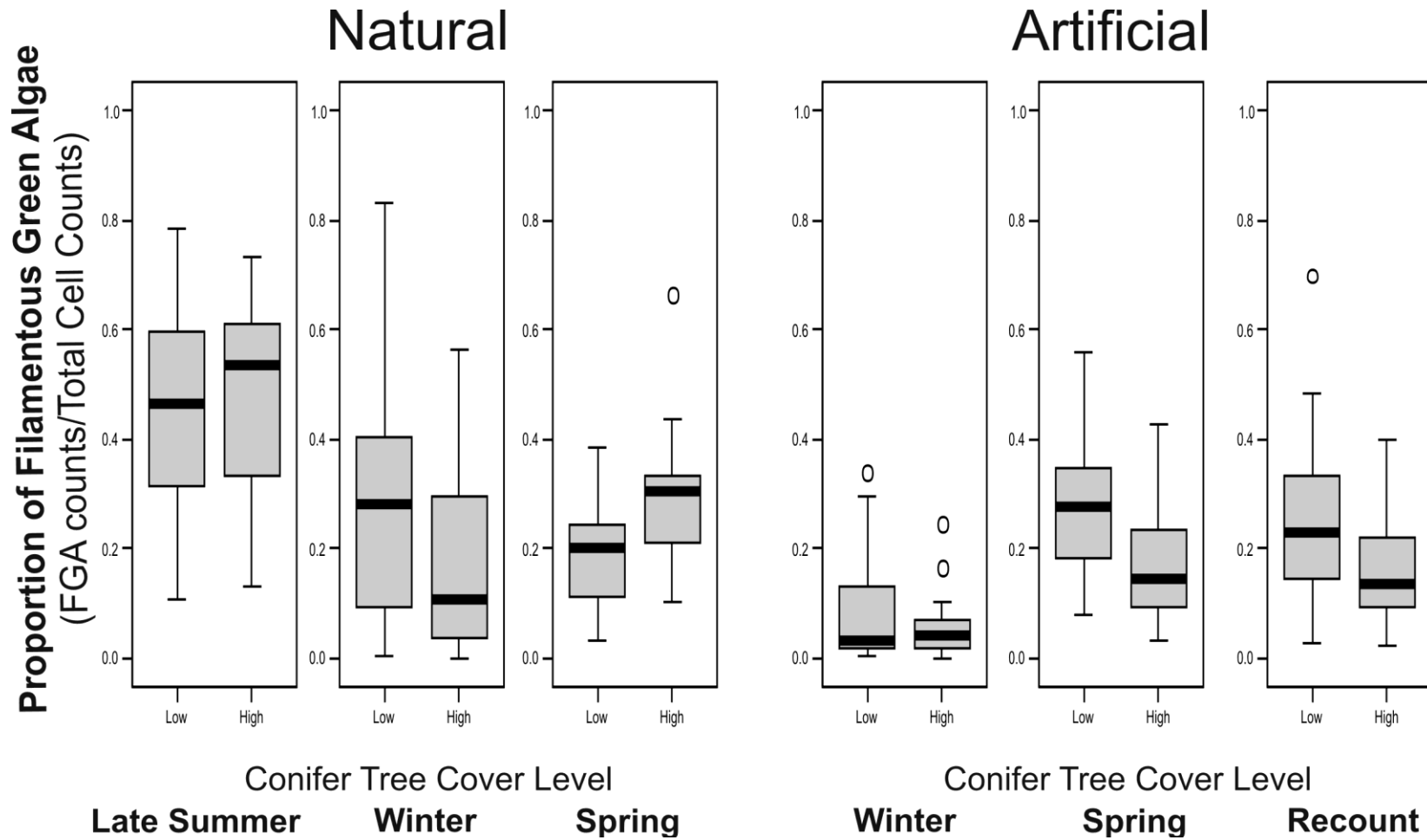
Figure 23. Proportion of filamentous green algae (FGA) as a function of conifer cover. There were no significant differences in FGA counts between sites with low and high conifer cover (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers). There was no consistent trend in FGA by season and substrate.
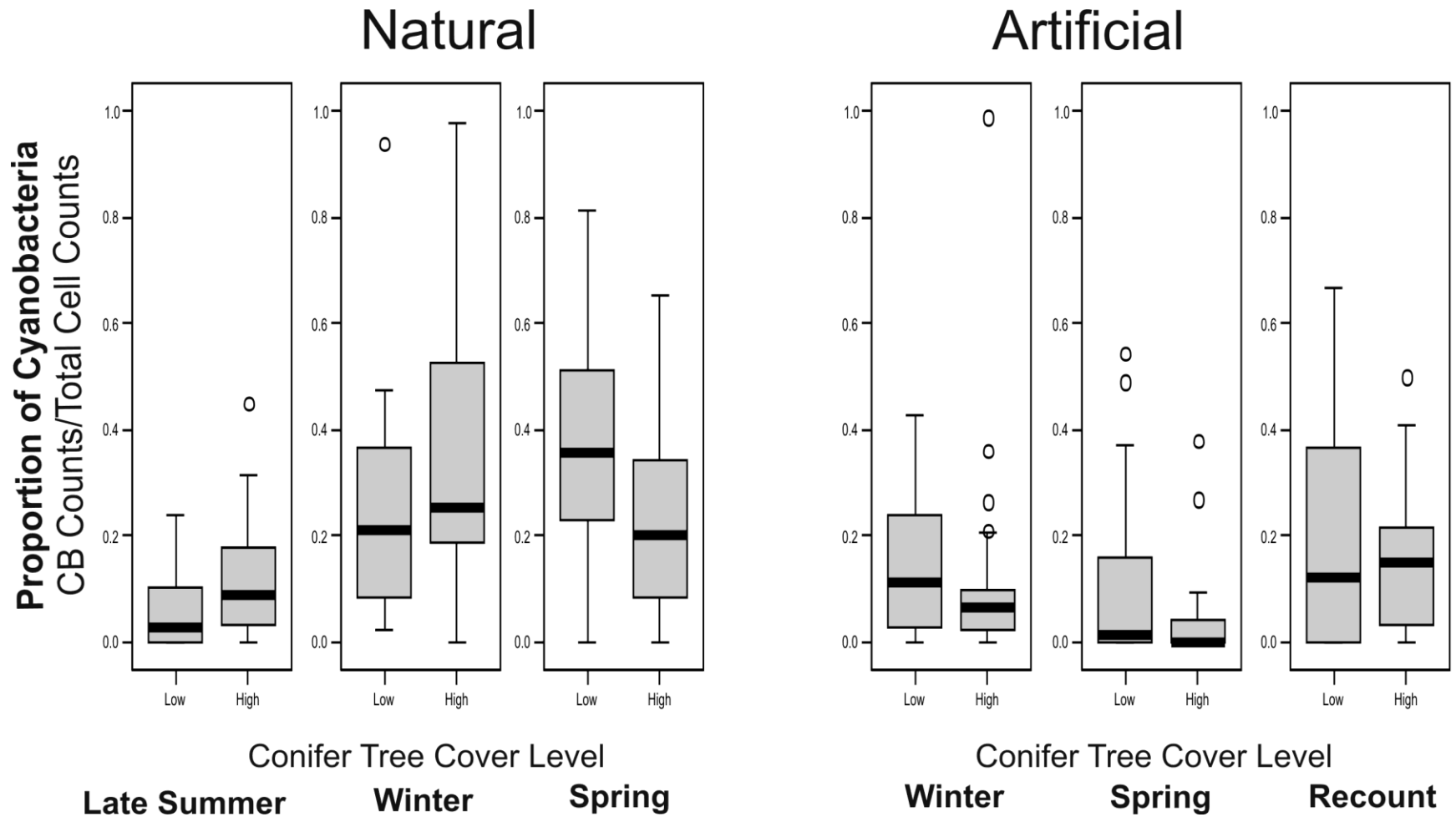
Figure 24. Proportion of cyanobacteria (CB) as a function of conifer cover. There were no significant differences in CB between sites with low and high conifer cover during any season (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).

Natural

Artificial

Figure 25. Proportion of diatoms (DT) as a function of conifer cover. Diatoms tended to be more abundant from all substrates at sites with high conifer cover from during all seasons except natural substrates during the late summer. No differences were significant however (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
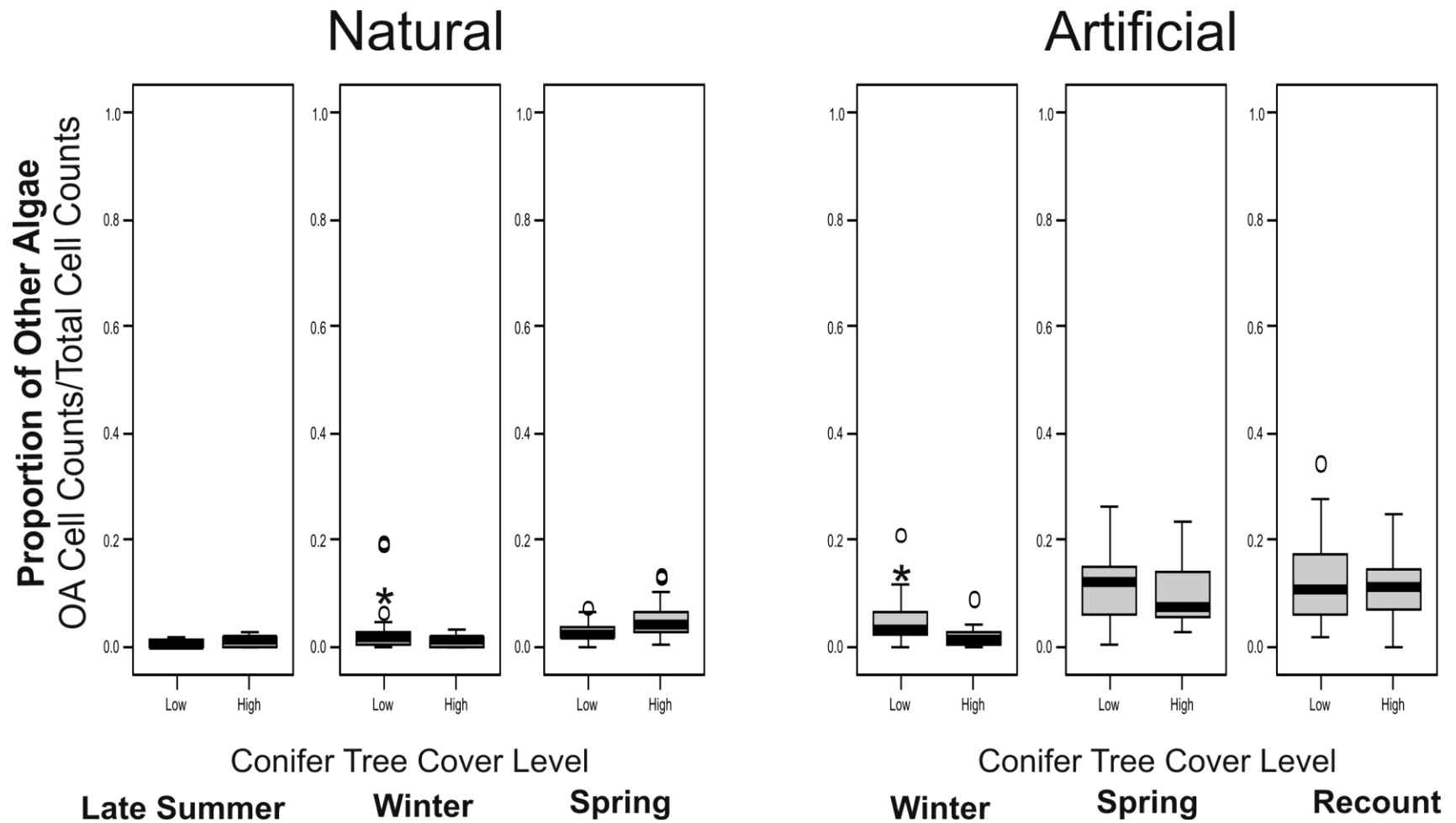
Figure 26. Proportion of other algae (OA) as a function of conifer cover. OA tended to be more abundant at sites with low conifer cover, but only significantly higher on natural and artificial substrates during the winter (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
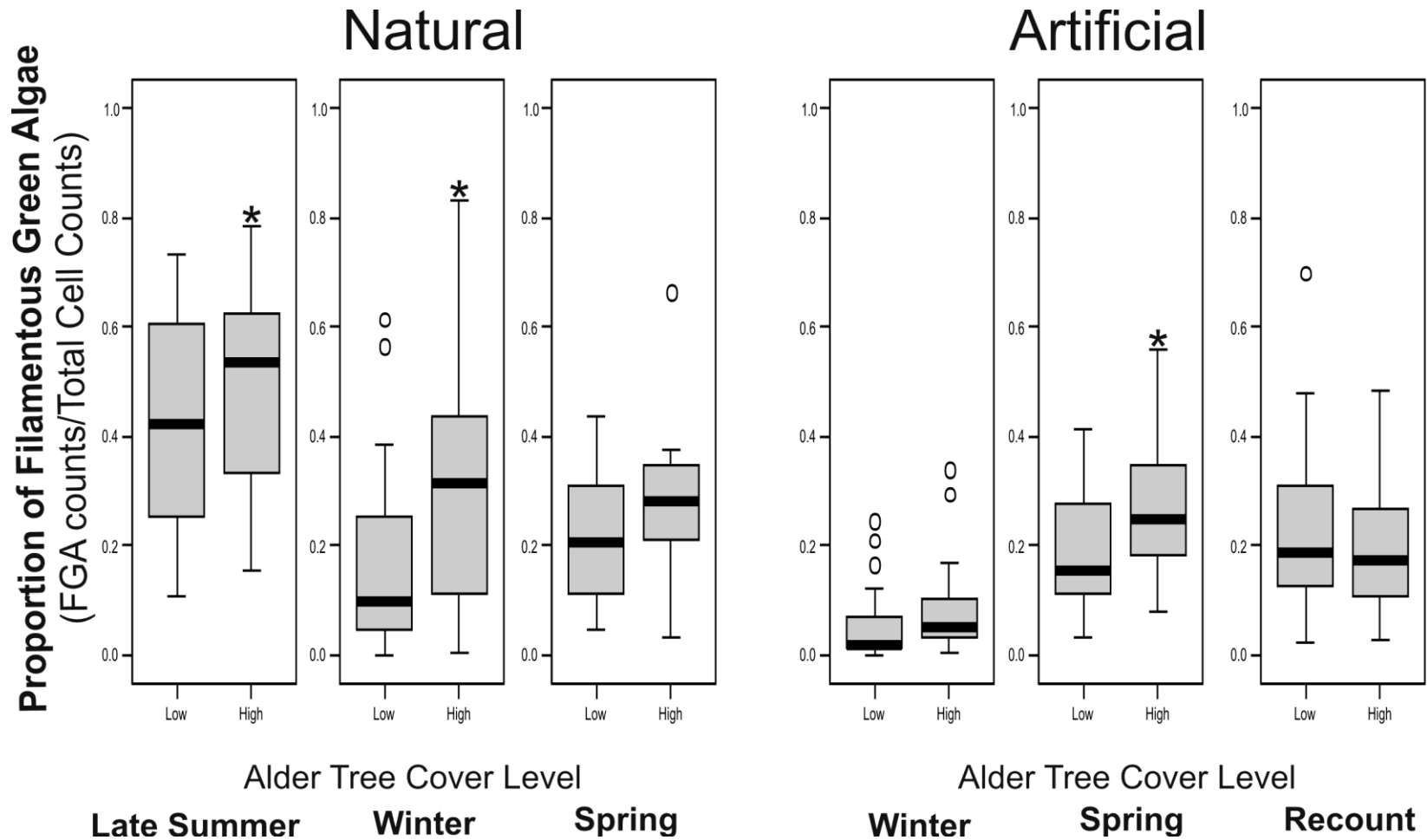
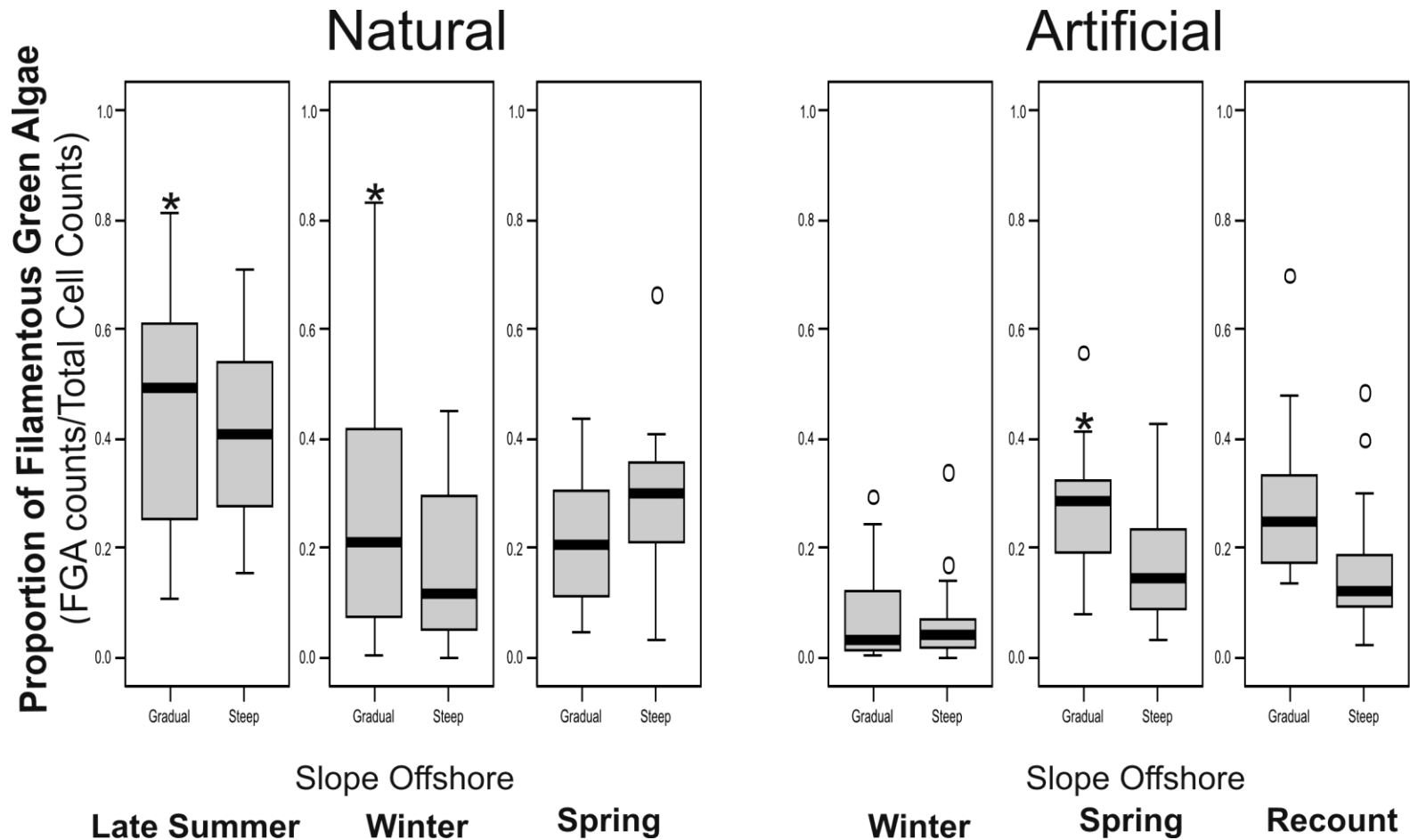Figure 27. Proportion of filamentous green algae (FGA) as a function alder cover. FGA were more common at sites with high alder cover for most substrates and seasons, and significantly for several (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
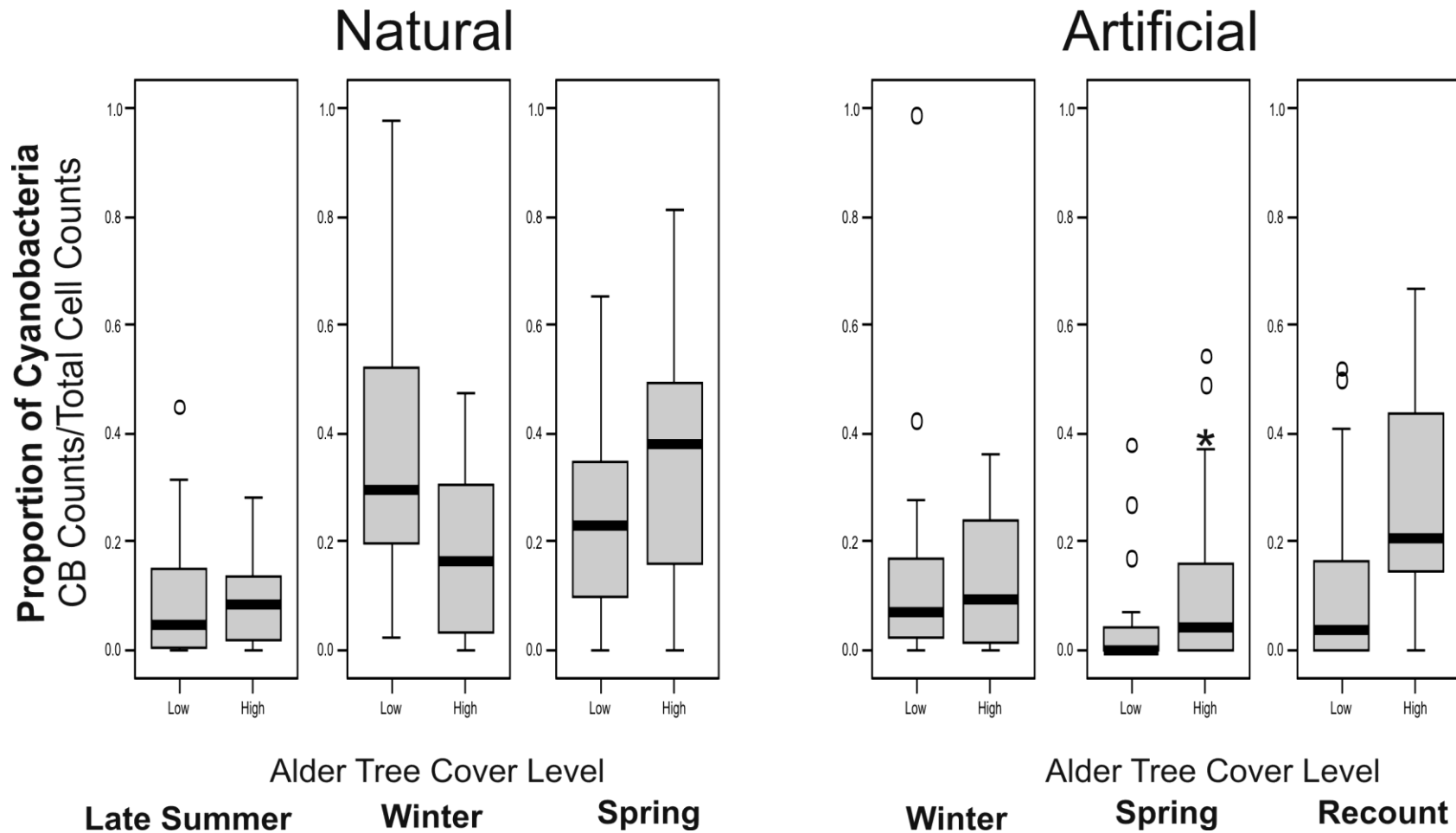
Figure 28.Proportion of cyanobacteria (CB) as a function of alder cover. CB tended to be lower on natural substrates with high alder cover except during the spring, but higher on artificial substrates with high alder cover. However, none of these trends were significant except for artificial substrates in the spring (General Linear Model(General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
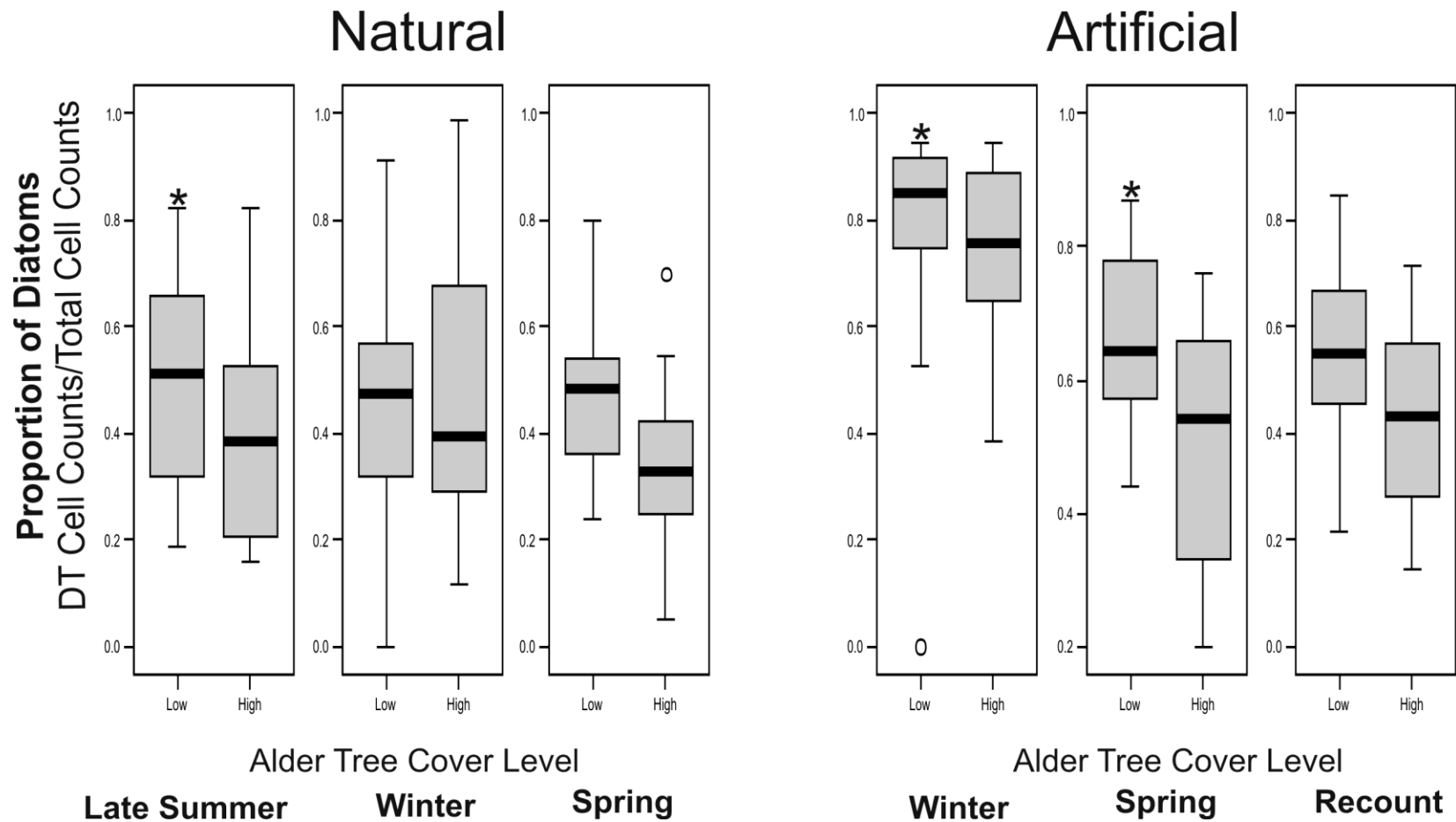
Figure 29. Proportion of diatoms (DTs) as a function of alder cover. DTs were consistently less abundant at sites with high alder cover and significantly so on natural substrates during the late summer, and artificial substrates during the spring (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).

Natural

Artificial

Proportion of Other Algae
OA Cell Counts/Total Cell Counts

Alder Tree Cover Level

Late Summer   **Winter**   **Spring**

Alder Tree Cover Level

Winter   **Spring**   **Recount**
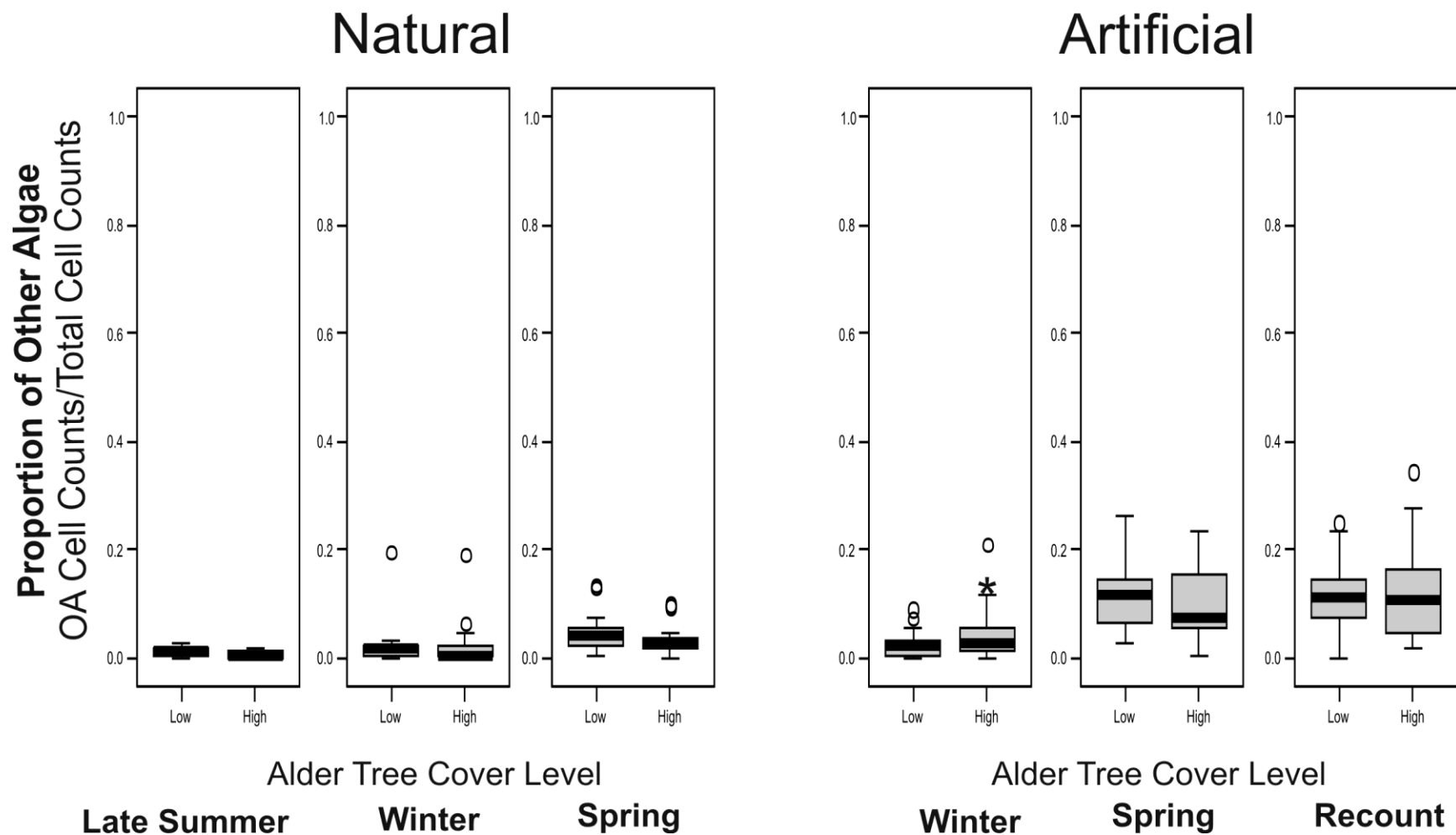
Figure 30. Proportion of other algae (OA) as a function of alder cover. Other algae tended to be more abundant at sites with higher alder tree cover, but only significantly on artificial substrates during the winter (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).

Development had little consistent effect on periphyton community composition except on natural substrates during the winter in which developed sites had more cyanobacteria (Figures 31-34). Developed sites consistently tended to have fewer diatoms than undeveloped sites but the trend was never significant.

The canonical correlation analysis showed that season was the independent variable most strongly correlated with variation in chlorophyll a, filamentous green algae, cyanobacteria, and AFDW (Table 4). Following closely and still significant in order of strength of correlation with variation in the dependent variables were alder cover, steepness of offshore slope, and conifer cover. Development was correlated with very little variation in the independent variables, and was not significant.
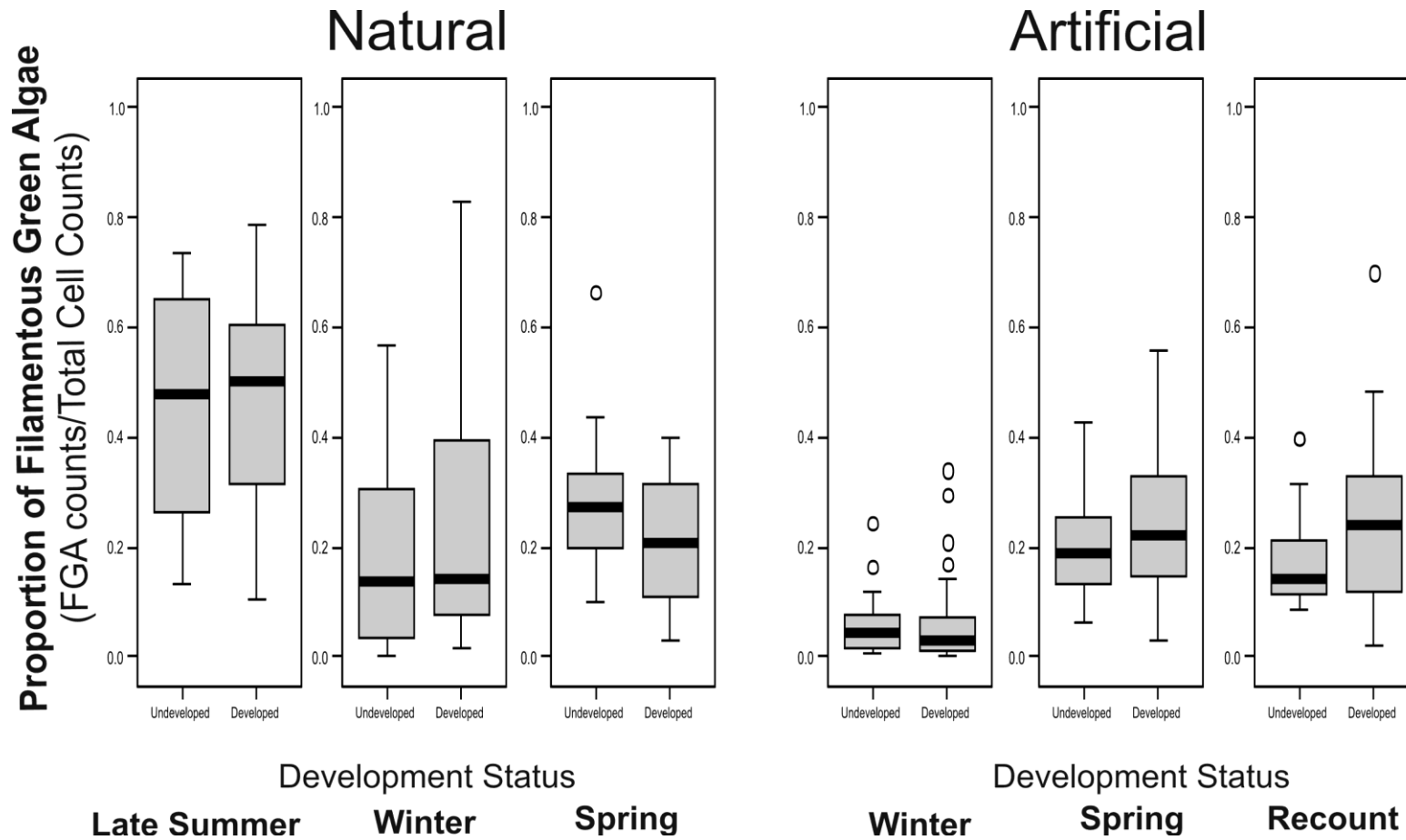
Figure 31. Proportion of filamentous green algae (FGA) as a function of development status. Filamentous green algae tended to be more abundant at developed sites for all substrates and seasons except natural substrates during the spring and artificial substrates during the winter. However, none of the differences were significant (General Linear Model. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
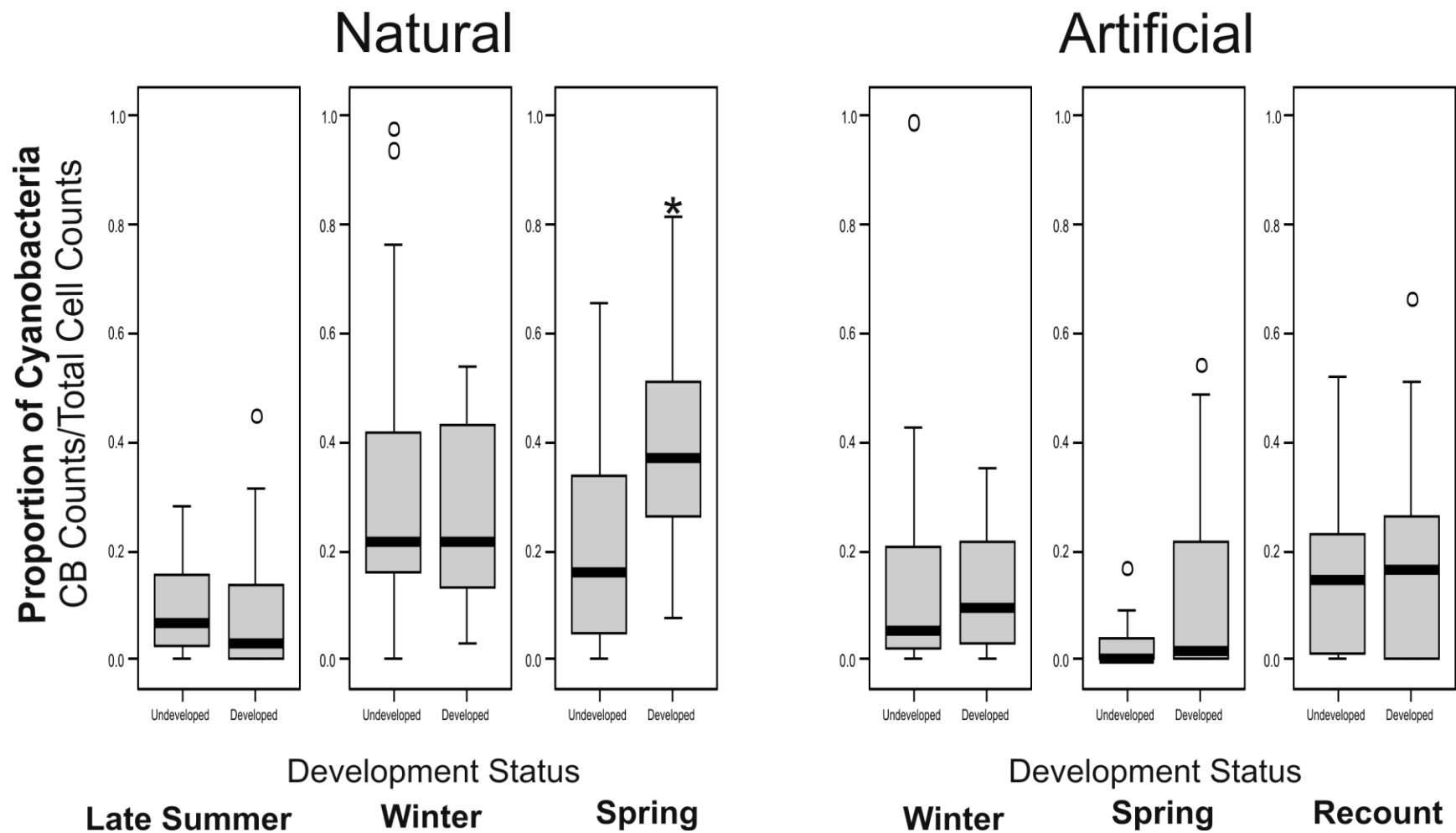
Figure 32. Proportion of cyanobacteria as a function of development status. Cyanobacteria tended to be higher at developed than undeveloped sites, but only significantly so on natural substrates during the spring (General Linear Model. Asterisks denote significant differences. Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
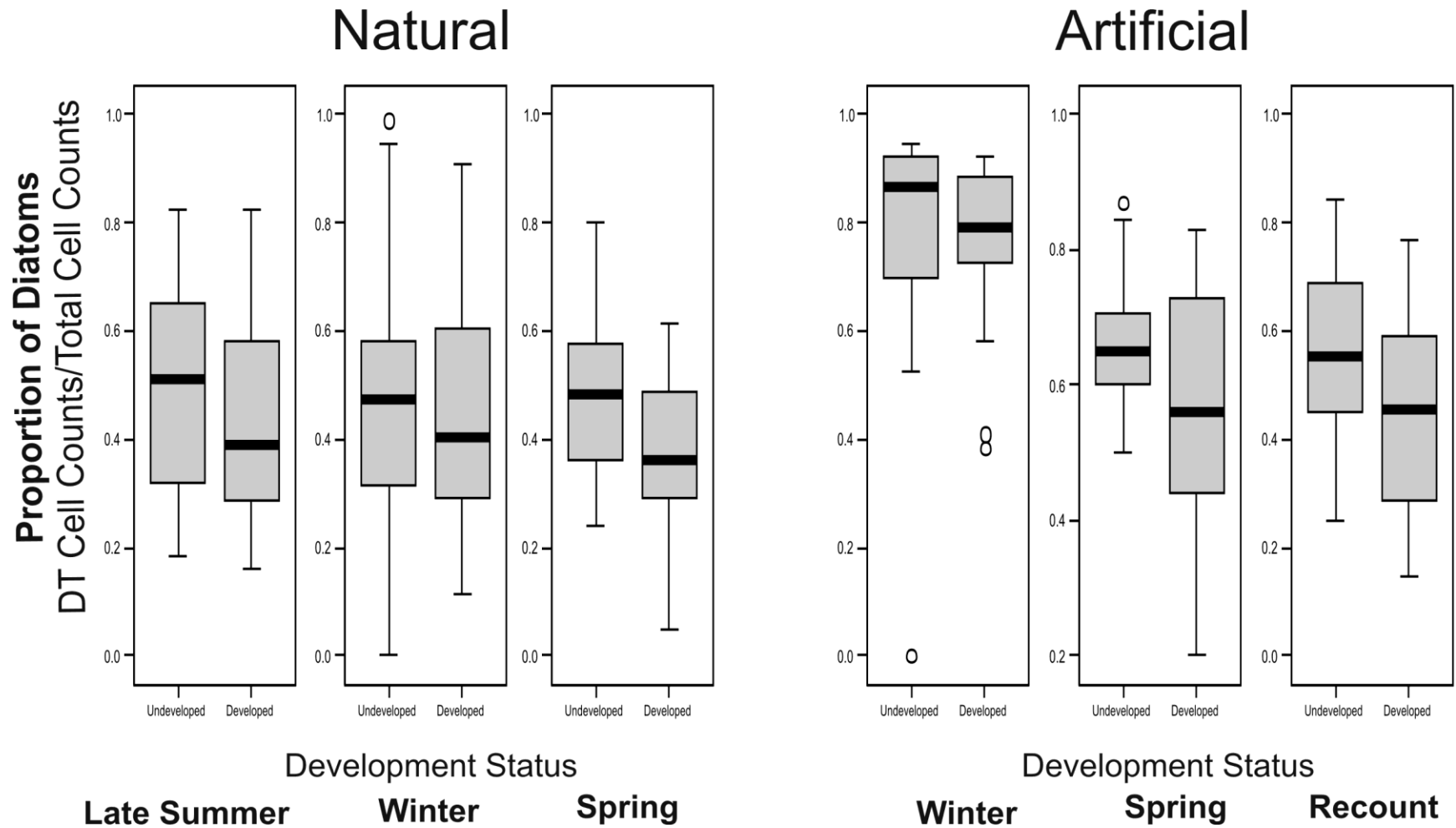
Figure 33. Proportion of diatoms (DT) as a function of development status. Although no differences were significant (General Linear Model), the consistent trend was toward lower DT abundance at developed sites (Boxplots: Box height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).
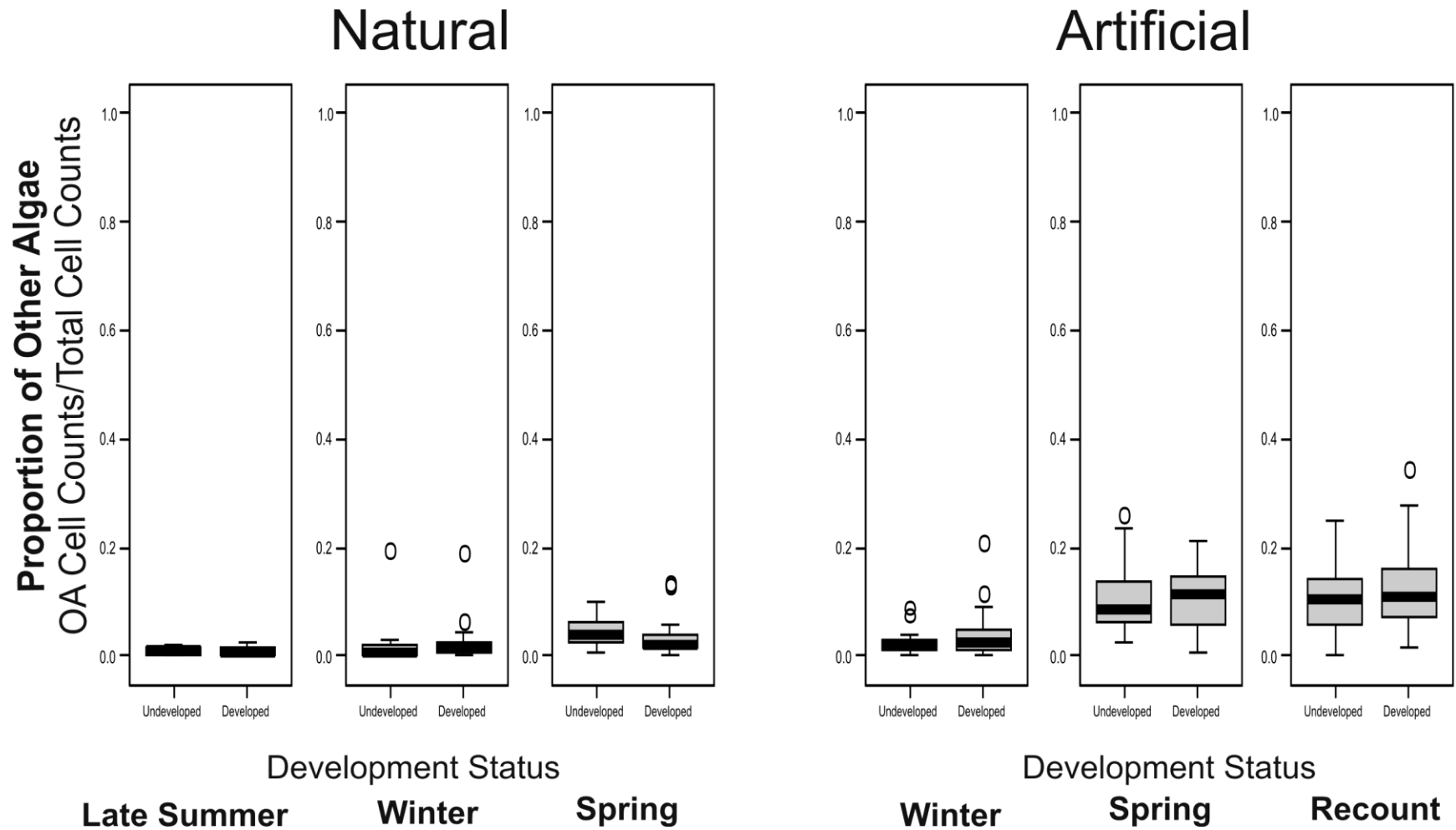
Figure 34. Proportion of other algae as a function of development status. The abundance of other algae was not significantly different for developed and undeveloped sites on any of the substrates during any of the seasons (General Linear Model. Boxplots: Box

height=interquartile range. Central band=median. Error bars=range excluding outliers. O=outliers).

Table 4 Canonical analysis

Component	Canonical Correlations	Wilk's Test	Cross Loadings for Independents					
			Alder Cover	Variation Explained	Offshore Slope	Variation Explained	Conifer Cover	Variation Explained
1	0.671	P<0.001, df=30	-0.357	12.70%	0.319	10%	-0.194	9%
2	0.625	P<0.001, df=20	N/A	N/A	-0.306	9%	-0.357	12.70%
			Cross Loadings for Dependents					
Component			Chlorophyll a	Variation Explained	Other Dependents	Variation Explained	Cyanobacteria	Variation Explained
1			-0.391	15%	Fil Green Algae=-0.530	53%	0.349	12%
2			0.380	14%	AFDW=-0.500	25%	0.338	11%

DISCUSSION

Human development sites along lakeshores, and especially septic systems, have in the past been associated with eutrophication in deep oligotrophic lakes (Goldman, 1988). Previous studies on Lake Crescent have also suggested that nutrients from lawns or failing septic systems associated with developed sites may be responsible for algae blooms (Meyer and Fradkin, 2002, Rosenberger et al., 2008). Therefore it was expected that similar results would be obtained in this study. However, the results of this study suggest a bit more nuanced picture. Although development status may indeed have an influence on the pattern of periphyton growth along the shore, this study suggests that, at least for these representative sites along Lake Crescent, other more natural factors may have a stronger influence on algal growth.

Lake Crescent is fairly unique among lakes due to its depth and moderate development so comparison to previous studies is difficult. Perhaps the closest comparison is Lake Tahoe, located on the border between California and Nevada. Lake Tahoe experienced a eutrophication event during the 1960's and 1970's. Studies carried on during this time showed that the eutrophication was directly linked to the development of houses and casinos along the lakeshore during that time (Goldman 1988). However, there are some important differences between Lake Crescent and Lake Tahoe. While Lake Tahoe has water retention time of 700 years Lake Crescent's retention is relatively short at 12 years (Goldman 1988, Meyer and Fradkin 2002). Also, Lake Tahoe is much deeper with a maximum depth of 505 m and an average depth of 313 m.

Possibly one of the most important characteristics that distinguishes the two lakes, however, is the amount and density of development along the shore. Lake Crescent has comparatively little development along its shoreline. Since the lake is now almost wholly within a national park, additional development will not continue. In addition, any privately owned tracts of land that go up for sale are purchased by Olympic National Park whenever possible (Fradkin, Personal communication). Perhaps it is because of these measures that Lake Crescent has remained as pristine as it is. Lake Tahoe, on the other hand, saw continued development at the time of its eutrophication problems. However, studies resulted in complete sewage export from the Lake Tahoe basin, decreasing the rate of eutrophication.

Effects of Site Characteristics on Periphyton Growth

The strongest and most consistent factor which differentiated sites was the offshore slope. Sites with shallow offshore slopes had consistently more biomass and a higher percent of filamentous green algae while sites with steep offshore slopes tended to have less biomass and a higher percent of diatoms. This result is not a result of differences in depth *per se* because samples at all sites were taken from 0.5 and 1 m depths. Instead, the differences were associated with the steepness of the slope itself. There are several possible reasons for this. Materials near the lake shore are disturbed by wave action along the shoreline. Organic nutrients, which have a density comparatively close to that of water, are likely easily suspended by this wave action. With a steep slope it would be easy for this material to move slightly offshore and sink down into water too deep to influence the nearshore periphyton. In beaches with shallow slopes, on the other hand, material would have to move much farther offshore before it could sink down to

unavailable depths. In this case, the higher periphyton growth on shallow shores would be due to greater availability of nutrient-laden organic material at those sites. Alternatively, water along shallow-sloped shores may be more strongly exposed to solar heating and less easily diluted by cooler water from below than is water from steeper shorelines. Increased thermal stratification on warm, calm days may increase average temperature in these habitats and stimulate increased green algal growth. The diatom groups most common in this study (Wehr and Sheath, 2003), on the other hand, are generally associated with colder water and the enhanced presence of diatoms at sites with steep slopes may indicate the greater influence of colder, deeper water at those sites.

The factor that seemed to have the second strongest impact on algae growth and community composition was the amount of alder tree cover. Sites with more alder tree cover tended to have higher biomass as well as more filamentous green algae. This is not surprising since alder trees can fix nitrogen and their leaves have higher nitrogen content than do other deciduous trees (Goldman, 1961, Lopez et al., 2001) and it has been shown that alder trees are largely responsible for differences in available nitrogen in northwest streams (Volk 2004, Compton et al. 2003, Volk et al. 2003, Sigleo et al. 2010). Lake Crescent is thought to be nitrogen limited (Washington State Department of Ecology, 1998, Meyer and Fradkin, 2002) so any increase in nitrogen input may have a fairly large effect on periphyton growth in the immediate area since periphyton may pick up many nutrients entering by way of stream or ground water rather quickly (Loeb and Goldman, 1979, Biggs, 1989).

Conifer cover had less effect than alder did. While there were a few significant differences between sites with high and low conifer cover, they were possibly due to

reduced sunlight reaching the water rather than nutrient loading since conifer needles have much lower nitrogen levels and take longer to break down (Lopez et al., 2001).

The biggest surprise in this study was the general lack of correlation between human development sites, all of which had septic systems, and periphyton biomass and community composition. Development status and the presence of failing septic systems would seem to be reasonable explanations for the observed increase in algae blooms in Lake Crescent, but since this study found no connection between development and periphyton biomass or community composition the question remains as to why it seems the blooms are getting worse.

Bunting et al., (2010) found that spatial and temporal variation in the nitrogen content of oligotrophic mountain lakes was strongly influenced by terrestrial subsidies of nitrogen in dissolved organic matter (DOM) to the lakes. They concluded that climate-related shifts in runoff and in the type and extent of vegetation in the lakes' catchment basins due to a shift in whether or not the lake was alpine or subalpine had strong effects on N input to the lakes. While Lake Crescent is well below the tree line, changes in overall vegetative cover may still have an impact. Increases in rainfall and temperature allowing for more vegetative growth (Bunting et al., 2010) and clearings due to logging which are associated with an increase of alders (Deal et al., 2004), could increase nutrient input. If this is the case in Lake Crescent, then it can be expected that algae blooms may continue if climate continues to warm or if alders become more widespread in the basin.

A Note on Collection Methods

To date this is the first study to successfully use artificial substrates for periphyton growth in Lake Crescent. Previous attempts had failed to produce any substantial periphyton growth on artificial substrates (Fradkin, personal communication). In this study there were quite a few significant differences in both community composition and biomass between natural and artificial substrates (Tables 5-7). However, the general trends of the natural and artificial substrates were mostly the same. It is possible that the natural substrates had longer time to collect debris or had a rougher surface that allowed the algae to attach or stick to the substrate better. In addition, the artificial substrates used also had more impermeable surface area than the natural substrate which might have prevented as much nutrients from seeping up from underneath them.

An important observation made during the collection of periphyton was that when the substrates were raised to the surface much of the filamentous green algae that could be seen floating just off the bottom of the lake tended to wash off from both natural and artificial substrates and be lost to analysis. This could be a possible source for some of the differences between results of this study and the previous study in which a double syringe periphyton sampler was used without raising the substrate from the bottom (Rosenberger et al., 2008). It is possible that creating a different artificial substrate or modifying collection techniques to ensure efficient collection of these poorly attached or unattached algae could eliminate this problem. In general, I found good algal growth on the artificial substrates. Since artificial substrates are initially algae-free, have standardized surfaces, and can be readily scraped clean at any time, they may give a better indication of seasonal patterns of algal growth and colonization.

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Table 5 T-test comparison of the effect of growth substrate on productivity. Value stated for each group=median. N=sample size. P=probability of the null hypothesis. t=t statistic for significant difference between groups. All groups were significantly different at the 0.05 conifdence level.

	Natural Substrates		Artificial substrates		
	Chlorophyll a (mg/m^2)	N	Chlorophyll a(mg/m^2)	N	T-test Results
Winter	5.8	48	6.8	39	P=0.018 t=2.4 df=49
Spring	11.8	41	8	43	P<0.001 t=9.2 df=40
	AFDW (mg/m^2)	N	AFDW (mg/m^2)	N	T-test Results
Winter	6700	38	1760	41	P<0.001 t=10.6 df=37
Spring	7000	39	8560	42	P<0.001 t=-6.5 df=56

Table 6 One-way ANOVA comparison with a Tukey post-hoc of the effect of algae growth substrate during the winter. Value stated for each group = median. N = sample size. F= f statistic for significant difference between the groups. P = probability of the null hypothesis. Groups significantly different at the 0.05 confidence level as determined by the Tukey test are printed in boldface except those labeled with an asterisk which are not significantly different from each other.

	Natural	N	Artificial	N	Recount	N	df	F	P
Filamentous Green	0.23	38	0.19	45	0.18	41	2	0.39	0.681
Cyanobacteria	**0.27**	41	**0.00**	45	**0.15**	41	2	19.60	0.000
Diatoms	**0.39**	41	***0.61**	45	***0.49**	41	2	11.90	0.000
Other	**0.03**	41	***0.10**	45	***0.11**	41	2	24.76	0.000

Table 7 T-test results of the comparison of the effect of algae growth substrate on periphyton community composition during the winter. Value stated for each group = median proportion. Sample size was 42 for natural substrates and 41 for artificial substrates. Df = degrees of fredom. t = t statistic for significant difference between groups. P = probability of the null hypothesis. All groups were significantly different except fo other algae from natural and artificial substrates.

	Natural Substrate	Artificial Substrate	T-test Results
Filamentous Green	0.14	0.04	P<0.001 t=4.6 df=65
Cyanobacteria	0.22	0.07	P<0.001 t=3.7 df=81
Diatoms	0.45	0.80	P<0.001 t=-5.8 df=81
Other	0.01	0.03	P=0.215 t=-1.3 df=81

The biggest drawback to this study was the available sampling sites. Most of the southern shore of Crescent Lake is bordered by a highway and it was difficult to find undeveloped sites that would have matched any developed sites with the same exposure. In addition, most of the properties that were owned by Olympic National Park were along the northern shore, limiting the study to only the northern shore of the lake. Therefore, if any important changes occurred along the southern shore, they were not accounted for. However, the steep shorelines and high ridges to the south of the lake cause the southern shoreline to be shaded more than the northern shoreline is. According to these factors, the most likely place for algal growth should be along the northern shoreline, which this study tested. Some algal blooms have been observed along the south shoreline (Fradkin personal communication) but these have been around heavily used resorts and boat launches in the Barnes Creek area, and their cause has yet to be determined.

As previously mentioned in the results, the sites chosen were not as closely matched to each other for tree cover as was initially planned. This was also a result of the limited number of developed sites, as well as the difficulty of accessing undeveloped sites along the shore and visually assessing approximate tree cover before making actual measurements. However, because as a whole the developed and undeveloped sites did not differ significantly in regards to tree cover, this is not likely to have changed the results.

In conclusion, this study found that natural factors, specifically offshore slope and alder cover, were more strongly associated with differences in periphyton growth and community composition than development was along the shore of Lake Crescent. My conclusion is that while anthropogenic factors such as seepage from septic systems at developed sites may be affecting algal growth in the lake, at this time the natural factors

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of offshore slope and alder cover are having a stronger effect. In addition, I propose that

although some modification to the artificial substrates would be beneficial, using artificial

substrates may be a useful tool in any further periphyton studies in Lake Crescent.

ACKNOWLEDGMENTS

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APPENDIX

Table I with GLM (General Linear Model) comparison of the effect of offshore slope, conifer cover, alder cover, and development on periphyton ash-free dry weight (AFDW), chlorophyll a content (Chl a) , and the composition by count of the algal periphyton community. Value stated for each group = median. A and B designate the two groups being compared. For both tree cover categories A=high cover and B=low cover. For slope A=gentle offshore slope and B= steep offshore slope. For Development A=undeveloped and B=developed. F = F statistic for significant difference between the groups. p = probability of the null hypothesis. Groups significantly different at the 0.05 confidence level are printed in boldface. Table Ib: winter data. Table Ic: Spring data.

	Offshore Slope	Sample Size	Statsitics	Conifer Cover	Sample Size	Statistics	Alder Cover	Sample Size	Statistics	Development	Sample Size	Statistics
AFDW												
A	2400	21	P=0.368	5250	20	P=0.074	1700	21	P=0.736	1600	19	P=0.362
B	2800	14	F=0.833	1600	15	F=3.407	5200	14	F=0.116	5250	16	F=0.855
Chla												
A	9.14	21	P=0.694	15.10	20	P= 0.240	5.26	21	P=0.182	5.26	19	P=0.051
B	13.54	14	F=0.157	4.43	15	F=1.421	14.96	14	F=1.853	14.41	16	F=4.058
AI												
A	225	23	N/A	225	23	N/A	239	24	P=0.099	280	27	P=0.336
B	288	15	df=0	288	15	df=0	393	14	F=2.877	225	11	F=0.953
Fil. Gr. Algae	N=24											
A	**0.55**	**21**	**P=0.020**	0.45	20	P=0.816	**0.42**	**21**	**P=0.010**	0.36	19	P=0.717
B	**0.37**	**14**	**F=0.231**	0.43	15	F=0.002	**0.54**	**14**	**F=0.292**	0.55	16	F=0.005
Cyanobacteria												
A	0.03	21	P= 0.299	0.02	20	P=0.326	0.03	21	P=0.427	0.06	19	P=0.356
B	0.08	14	F=1.112	0.07	15	F=0.030	0.05	14	F=0.019	0.01	16	F=0.026
Diatoms	N=24											
A	**0.40**	**21**	**P=0.031**	0.48	20	P=0.636	**0.53**	**21**	**P=0.002**	0.53	19	P=0.197
B	**0.52**	**14**	**F=0.165**	0.5	15	F=0.007	**0.39**	**14**	**F=0.365**	0.42	16	F=0.056
Other	N=24											
A	0.01	21	P=0.292	0.01	20	P=0.297	0.01	21	P=0.619	0.01	19	P=0.963
B	0.01	14	F=0.000	0.01	15	F=0.003	0.01	14	F=0.001	0.01	16	F=0.000

Table Ib Winter Data	Offshore Slope	Sample Size	Statistics	Conifer Cover	Sample Size	Statistics	Alder Cover	Sample Size	Statistics	Devlopement	Sample Size	Statistics
Nat.Sub AFDW												
A	**13389**	**19**	**P=0.034**	**13200**	**19**	**P=0.023**	**5400**	**23**	**P=0.010**	6200	18	P=0.105
B	**3600**	**15**	**F=4.932**	**1900**	**15**	**F=5.711**	**11800**	**11**	**F=6.112**	7150	16	F=1.496
Art. Sub AFDW												
A	1740	22	P=0.408	1640	23	P=0.447	1760	29	P=0.282	1760	18	P=0.505
B	1760	19	F=0.701	1800	18	F=0.592	1420	12	F=1.198	1760	23	F=0.455
Nat. Sub Chla												
A	**10.41**	**19**	**P<0.001**	10.94	19	P=0.224	**6.68**	**23**	**P<0.001**	6.70	18	P=0.317
B	**4.54**	**15**	**F=19.832**	3.81	15	F=1.523	**16.02**	**11**	**F=23.823**	8.71	16	F=1.026
Art. Sub Chla												
A	**4.17**	**16**	**P=0.014**	7.30	18	N/A	5.93	21	P=0.123	4.81	15	P=0.558
B	**7.34**	**23**	**F=65.099**	5.66	21	df=0	7.30	18	F=24.102	7.30	24	F=3.375
Natural Sub AI												
A	756	21	N/A	756	21	N/A	519	25	P=0.974	540	28	P=0.854
B	480	17	df=0	480	17	df=0	756	13	F=0.001	587	10	F=0.034
Artificial Sub AI												
A	524	17	P=0.090	471	23	N/A	466	23	P=0.663	**502**	**12**	**P=0.003**
B	256	18	F=3.095	282	12	df=0	262	12	F=0.194	**236**	**23**	**F=10.862**
Nat. Sub Fil. Gr.												
A	**0.20**	**19**	**P=0.018**	0.26	19	P=0.930	**0.09**	**23**	**P=0.007**	0.13	18	P=0.976
B	**0.12**	**15**	**F=6.178**	0.08	15	F=0.008	**0.31**	**11**	**F=8.218**	0.19	16	F=0.001
Art. Sub Fil. Gr.												
A	0.03	18	P=0.405	0.03	20	P=0.238	0.02	24	P=0.203	0.05	18	P=0.975
B	0.04	23	F=0.710	0.04	21	F=1.445	0.05	17	F=1.689	0.03	23	F=0.001
Nat. Sub Cyano												
A	0.22	19	P=0.803	0.22	19	P=0.205	0.30	23	P=0.122	0.26	18	P=0.881
B	0.3	15	F=0.063	0.30	15	F=1.672	0.17	11	F=2.519	0.25	16	F=0.023
Art. Sub Cyano												
A	**0.11**	**18**	**P=0.007**	**0.11**	**20**	**P=0.007**	0.07	24	P=0.254	0.05	18	P=0.677
B	**0.06**	**23**	**F=3.591**	**0.07**	**21**	**F=3.591**	0.09	17	F=1.349	0.10	23	F=0.177
Nat. Sub Diat												
A	**0.41**	**19**	**P=0.014**	0.48	19	P=0.230	0.48	23	P=0.395	0.48	18	P=0.649
B	**0.47**	**15**	**F=6.713**	0.44	15	F=1.494	0.38	11	F=0.742	0.41	16	F=0.210
Art. Sub Diat												
A	**0.76**	**18**	**P=0.005**	0.77	20	P=0.457	**0.85**	**24**	**P=0.047**	0.87	18	P=0.670
B	**0.88**	**23**	**F=9.158**	0.88	21	F=0.566	**0.76**	**17**	**F=4.255**	0.79	23	F=0.185
Nat. Sub Other												
A	0.02	19	P=0.345	**0.02**	**19**	**P=0.012**	0.01	23	P=0.536	0.01	18	P=0.486
B	0.01	15	F=0.916	**0.01**	**15**	**F=7.043**	0.005	11	F=0.391	0.02	16	F=0.497
Art. Sub Other												
A	**0.04**	**18**	**P=0.023**	**0.03**	**20**	**P=0.009**	**0.02**	**24**	**P=0.013**	0.02	18	P=0.178
B	**0.02**	**23**	**F=5.698**	**0.01**	**21**	**F=7.677**	**0.03**	**17**	**F=6.977**	0.03	23	F=1.897

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Table IC Winter Data	Offshore Slope	Sample Size	Statistics	Conifer Cover	Sample Size	Statistics	Alder Cover	Sample Size	Statistics	Devlopement	Sample Size	Statistics
Nat. Sub. AFDW												
A	10700	20	P=0.085	10350	18	P=0.151	8600	19	P=0.095	7150	16	P=0.375
B	2750	12	F=3.175	5000	14	F=2.166	5800	13	F=2.959	9100	16	F=0.811
Art. Sub. AFDW												
A	13660	22	P=0.157	8880	22	P=0.358	**9700**	**25**	**P=0.013**	9270	18	P=0.075
B	4020	20	F=2.094	6060	20	F=0.870	**5560**	**17**	**F=6.180**	5280	24	F=3.386
Nat. Sub. Chla												
A	**23.87**	**20**	**P=0.001**	23.57	18	P=0.339	11.75	19	P=0.355	11.01	16	P=0.224
B	**6.94**	**12**	**F=12.100**	9.35	14	F=0.941	18.16	13	F=0.881	20.03	16	F=1.535
Art. Sub. Chla												
A	10.00	20	P=0.883	9.75	21	P=0.613	11.21	25	P=0.079	9.04	20	P=0.976
B	7.85	23	F=0.022	8.01	22	F=0.260	7.32	18	F=3.262	8.01	23	F=0.001
Nat. Sub. AI												
A	225	23	P=0.682	225	23	N/A	**239**	**24**	**P=0.014**	**280**	**27**	**P=0.029**
B	288	15	F=0.171	288	15	df=0	**393**	**14**	**F=6.833**	**225**	**11**	**F=5.255**
Art. Sub. AI												
A	1003	20	P=0.975	857	25	N/A	1061	23	P=0.150	**1303**	**18**	**P=0.038**
B	690	19	F=0.001	833	14	df=0	659	16	F=2.179	**624**	**21**	**F=4.716**
Nat. Sub. Fil. Gr.												
A	0.21	20	P=0.947	0.22	18	P=0.838	0.21	19	P=0.134	0.30	16	P=0.140
B	0.30	12	F=0.005	0.30	14	F=0.043	0.32	13	F=2.359	0.21	16	F=2.291
Art. Sub. Fil. Gr.												
A	**0.29**	**21**	**P=0.002**	0.28	22	P=0.854	**0.16**	**27**	**P=0.046**	0.19	21	P=0.348
B	**0.15**	**24**	**F=10.743**	0.15	23	F=0.034	**0.25**	**18**	**F=4.254**	0.22	24	F=0.904
Recount Fil. Gr.												
A	**0.25**	**20**	**P=0.034**	0.23	20	P=0.169	0.19	25	P=0.255	0.14	20	P=0.051
B	**0.12**	**21**	**F=4.871**	0.14	21	F=1.981	0.17	16	F=1.344	0.03	21	F=4.108
Nat. Sub. Cyano.												
A	0.34	20	P=0.452	0.34	18	P=0.286	0.27	19	P=0.994	**0.20**	**16**	**P=0.042**
B	0.26	12	F=0.580	0.22	14	F=1.178	0.37	13	F=0.000	**0.34**	**16**	**F=4.469**
Art. Sub. Cyano.												
A	**0.02**	**21**	**P=0.003**	0.01	22	P=0.606	**0.00**	**27**	**P<0.001**	0.00	21	P=0.912
B	**0.00**	**24**	**F=10.066**	0.00	23	F=0.271	**0.04**	**18**	**F=16.259**	0.02	24	F=0.012
Recount Cyano.												
A	0.05	20	P=0.476	0.12	20	P=0.083	**0.04**	**25**	**P=0.001**	0.15	20	P=0.181
B	0.15	21	F=0.521	0.15	21	F=3.186	**0.21**	**16**	**F=14.552**	0.17	21	F=1.863
Nat. Sub. Diat.												
A	0.46	20	P=0.978	0.38	18	P=0.290	0.49	19	P=0.251	0.48	16	P=0.202
B	0.37	12	F=0.001	0.45	14	F=1.156	0.32	13	F=1.366	0.36	16	F=1.696
Art. Sub. Diat.												
A	**0.56**	**21**	**P<0.001**	0.56	22	P=0.487	**0.65**	**27**	**P=<0.001**	0.65	21	P=0.840
B	**0.70**	**24**	**F=35.569**	0.69	23	F=0.494	**0.54**	**18**	**F=30.036**	0.56	24	F=3.160
Recount Diat.												
A	**0.45**	**20**	**P=0.004**	0.44	20	P=0.859	**0.55**	**25**	**P=0.008**	0.55	20	P=0.190
B	**0.54**	**21**	**F=9.743**	0.55	21	F=0.032	**0.43**	**16**	**F8.039**	0.46	21	F=1.792
Nat. Sub. Other												
A	0.02	20	P=0.316	0.02	18	P=0.193	0.04	19	P=0.863	0.04	16	P=0.841
B	0.04	12	F=1.035	0.04	14	F=1.768	0.03	13	F=0.030	0.02	16	F=0.041
Art. Sub. Other												
A	0.13	21	P=0.492	0.12	22	P=0.867	0.12	27	P=0.325	0.09	21	P=0.628
B	0.08	24	F=0.482	0.08	23	F=0.29	0.08	18	F=0.994	0.12	24	F=0.238
Recount Other												
A	0.12	20	P=0.237	0.11	20	P=0.530	0.11	25	P=0.382	0.11	20	P=0.094
B	0.11	21	F=1.448	0.11	21	F=0.404	0.11	16	F=0.784	0.11	21	F=2.972