

TROPHIC CASCADE EFFECTS ON ALGAE BLOOMS IN WASHINGTON STATE



Ecology Grant Agreement WQALG-2019-LibWSD-00018

**Prepared for
Liberty Lake Sewer and Water District**

**Prepared by
Herrera Environmental Consultants, Inc.**



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**Prepared for
Liberty Lake Sewer and Water District
22510 E. Mission Ave
Liberty Lake, Washington 99019**

**Prepared by
Herrera Environmental Consultants, Inc.
2200 Sixth Avenue, Suite 1100
Seattle, Washington 98121
Telephone: 206-441-9080**

**Funded by
Washington State Department of Ecology
Freshwater Algae Program
Grant Number WQALG-2019-LibWSD-00018**

May 12, 2021

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ACKNOWLEDGEMENTS

This project was made possible by a grant to the Liberty Lake Sewer and Water District from the Washington State Department of Ecology Freshwater Algae Control Grant Program. Guidance on the data analysis procedures and review of the draft report was provided by the following members of the Technical Committee:

- Rob Zisette, Herrera Environmental Consultants
- BiJay Adams, Liberty Lake Sewer and Water District
- Jeremy Jenkins, Liberty Lake Sewer and Water District
- Lizbeth Seebacher, Washington Department of Ecology
- Randall Osborne, Washington Department of Fish and Wildlife
- Justin Spinelli, Washington Department of Fish and Wildlife
- Joan Hardy (retired), Washington Department of Health
- Daniel Schindler, University of Washington
- Mike Brett, University of Washington
- Tom Brattebo, Liberty Lake Resident
- Chris Knutson, King County Water and Land Resources Division
- Debra Bouchard, King County Water and Land Resources Division
- Marisa Burghdoff, Snohomish County Surface Water Management
- Jen Oden, Snohomish County Surface Water Management
- Isabel Ragland, Pierce Conservation District
- Jane Mountjoy-Venning, Thurston County Public Health and Social Services
- Drew Stang, Herrera Environmental Consultants

The literature review was conducted by Ellen Preece, Robertson-Bryan, Inc.

EXECUTIVE SUMMARY

The primary goal of this study was to determine whether trout stocking affects phytoplankton abundance and the occurrence of toxic cyanobacteria blooms in Washington lakes. A literature review was conducted that generated assumptions and hypotheses for using existing lake and trout stocking data to examine the potential for a trophic cascade effect of increased algae blooms and cyanobacteria toxin production from reduced zooplankton populations or increased nutrient supply caused by increased zooplankton grazing or nutrient inputs by trout stocking.

The trophic cascade effect of trout stocking in Washington lakes was evaluated by compiling existing water quality, cyanobacteria toxin, and trout stocking databases, and evaluating relationships between these variables. Two different data sets were used for this study that included Liberty Lake in eastern Washington and 88 western Washington lakes in Snohomish, King, Pierce, and Thurston counties. The Liberty Lake data set included 18 years of comprehensive water quality, phytoplankton, and zooplankton data provided by the Liberty Lake Sewer and Water District, and fish stocking data for trout and warmwater fish provided by the Washington Department of Fish and Wildlife (WDFW). The western Washington lakes dataset included at least 10 years of data from routine monitoring of water quality (field parameters, nutrients, and chlorophyll) provided by the counties, algae scum cyanotoxin concentration data available from the Washington Department of Ecology's Freshwater Algae Bloom Monitoring Program, and trout stocking provided by WDFW. These data were used to evaluate relationships among the key variables on a seasonal and annual basis using statistical analyses.

Liberty Lake data analysis included principle component analysis and multiple regression analysis. Key findings of the principle component analysis include:

- Liberty Lake exhibits standard trophic interactions with increased trout stocking decreasing zooplankton abundance and increasing phytoplankton biovolume.
- Stocking of legal-sized trout reduce zooplankton more than stocking of trout fry.
- Total phosphorus increases with trout stocking, possibly due to phosphorus in trout excretion or in increased phytoplankton biovolume.

Key findings of the Liberty Lake multiple regression analysis include:

- Significant correlations among parameters were only observed at the main lake NW station and not the shallow lake SE station, and most of those correlations were in the near surface samples (NWTOP).
- No significant correlations were observed using zooplankton parameters as the dependent variable, indicating that zooplankton populations are not principally controlled by trout stocking or the other parameters included in the multiple regression.

- Total cyanobacteria biovolume was positively correlated with trout stock mass at NWTOP, but no zooplankton or other phytoplankton parameters correlated with trout stock mass. Although these results indicate that trout stocking may stimulate cyanobacteria growth, there was not a clear indication that this was induced by a trophic cascade because of the lack of significant correlations between trout stock mass and zooplankton.
- Cyanobacteria and other phytoplankton groups were significantly positively correlated with total phosphorus and negatively correlated with orthophosphate at the NW station, reflecting the importance of phosphorus uptake by all types of algae in Liberty Lake.

For the western Washington lakes dataset, relationships between key variables were explored using multiple regression analyses of spring (April – June) and annual (April-October) datasets. The results showed extensive variability among parameters and between lakes. The only clear statistically significant relationships were between chlorophyll, total phosphorus, and water clarity, as would be expected from limnological principals.

Overall, no strong conclusion could be made regarding stocked trout inducing algae blooms or cyanotoxin production for the western Washington lakes. However, the analysis results indicate that algae blooms or cyanotoxin production may be significantly affected by trout stocking in some lakes. One limiting factor in this analysis is that trout stocking rates did not vary much between years for each lake. Significant correlations are difficult to detect without a wide range of values for each variable.

No changes in the trout stocking program are recommended at this time based on the findings of this study. Additional data collection and analysis would be needed to provide a scientific basis for changing the trout stocking program in Washington or a specific lake.

A next step in the data analysis could be to further investigate which lake characteristics are associated with the observed positive correlations between trout stocking and algae blooms. With a smaller sample size of six lakes showing significant positive correlations and seven lakes showing significant negative correlations, a more in-depth analysis could be conducted.

Additional analysis of the database compiled for this study could provide more meaningful results. In particular, the western Washington data could be analyzed further using different metrics associated with the trout size (e.g., legal versus jumbo sizes) and stocking timing (e.g., spring versus fall) with respect to immediate effects on chlorophyll-a, cyanotoxins, and nutrients. Additional independent variables could be included in the analysis such as lake size and depth, stratification stability, residence time, watershed to lake area ratio, and percent imperviousness or development of the watershed. Synthesizing results from the present study and additional analyses could be further analyzed using a decision tree to predict the effects of trout stocking relative to other factors on algae blooms and cyanobacteria toxin production. Factors controlling algae blooms and toxin production are complex and are expected to vary greatly between lakes with different physical, chemical, and biological characteristics. Resident fish and

aquatic macrophyte populations are examples of factors that likely impact algae blooms but are often not well characterized.

Lakes are highly dynamic ecosystems that can change significantly at daily time scales. The data sets used here do show many trends at large time scales, but sparse and averaged data sets do not accurately represent these dynamic systems. To fully understand the impacts of trout stocking, a more robust field study would be required. Trout are normally stocked in spring, which is typically the most dynamic period for plankton in lakes due to increasing temperatures and light for growth. Ideally, data for the key parameters identified in this study would be collected at higher frequencies before and after trout stocking, would include additional key parameters such as zooplankton species biomass, and would include more variation in trout stocking rates within each lake studied than typically occurs.

INTRODUCTION

The primary goal of this study was to determine whether trout stocking affects phytoplankton abundance and the occurrence of toxic cyanobacteria blooms in Washington lakes. The study approach was to compile existing water quality, toxic algae, and trout stocking databases and evaluate relationships between parameters. Two separate data sources were compiled and analyzed in comparison to fish stocking data. A detailed analysis of Liberty Lake, located in eastern Washington, was performed using an extensive historical water quality database that included phytoplankton and zooplankton species abundance data. In addition, trophic state parameter data were compiled from ambient monitoring programs for 96 lakes in western Washington.

Primary questions addressed in this study include:

- Does fish stocking significantly increase chlorophyll, nutrients, plankton species abundance/dominance, or toxic cyanobacteria blooms in Liberty Lake and other Washington lakes?
- What fish management or other factors are most highly correlated with cyanobacteria blooms?
- Do the observed effects depend on fish management variables (e.g., size/type of fish, number/frequency/biomass of fish added, or seasonal timing of additions), lake morphological variables (e.g., area, volume, mean depth), or lake water quality variables (e.g., trophic state, surface temperature, bottom dissolved oxygen)?

Three different tasks were defined to answer these questions. The first task was a literature review to summarize findings of relevant studies on the trophic cascade effects of fish management on phytoplankton in lakes to both guide the data analysis and to compare to results from this study. The second task involved analyzing data for Liberty Lake that have been collected over the past 18 years to evaluate trophic cascade effects of trout stocking on taxonomic groups of plankton species in the lake. The third task was to analyze data from other Washington lakes to evaluate trophic cascade effects on phytoplankton biomass and cyanotoxin concentrations. This report presents the results for each of these tasks, followed by a summary and conclusion that includes management recommendations.

This study was conducted by Herrera Environmental Consultants (Herrera) for the Liberty Lake Sewer and Water District (District) with funding from the Washington State Department of Ecology (Ecology) Freshwater Algae Control Grant Program. A technical review committee was formed to guide data analysis and review this report that included representatives of Herrera, District, Ecology, Washington Department of Fish and Wildlife (WDFW), Washington Department of Health, University of Washington, lake managers for counties providing data, and a Liberty Lake resident (see Acknowledgements).

LITERATURE REVIEW

A literature review was initially performed for this study and presented to the Technical Committee. A supplemental review was then performed for some additional papers recommended by the Technical Committee.

INITIAL REVIEW

The literature review involved compilation, review, and presentation of abstracts for 31 relevant publications on the trophic cascade effects of fish management on phytoplankton in lakes. A second bibliography of 33 publications on the general topic regarding trophic cascades was also compiled. The literature review findings, abstracts, and bibliographies are presented in Appendix A. The publications are made available along with this report.

The literature review findings are summarized separately for the following topics specifically related to trout stocking:

1. Survival, Growth, and Creel of Stocked Trout
2. Trout Diet and Size Class
3. Trout Stocking and Lake Food Webs
4. Trout Stocking and Nutrients

One outcome of the literature review was the discovery that there was a paucity of research available to support any of the four study topics. The few studies available in combination with the wide diversity of lake environments they represented likely hampered the potential for making definitive conclusions. The following are general summary statements of the key findings for each study topic:

Survival, Growth and Creel of Stocked Trout

Numerous factors affect the survival, growth, and catch rates (creel) of stocked trout, including: size class stocked, feeding competition with other fish, environmental conditions, fish strain, stocking density and season, and angler pressure. Of the rainbow trout fry that are stocked in lakes, 20 percent return to creel in western Washington compared to a 70 percent return in eastern Washington. The environmental conditions (e.g., temperature, acidity, dissolved oxygen) of western Washington lakes are not ideal for supporting the long-term survival of rainbow trout. This is one possible explanation for the lower creel in western Washington compared to eastern Washington. In western Washington lakes where environmental conditions are suboptimal, smaller fish generally do not support a put and take fishery. Anglers prefer fishing

for larger fish and therefore systems that stock larger fish (greater than 280 millimeters [mm]) recruit more anglers in western Washington.

Trout Diet and Size Class

Trout are opportunistic feeders that rely on a wide variety of prey from zooplankton and small insects to crayfish and smaller fish. In general, adult and juvenile trout rely on similar prey sources for their diets, but adult trout may have a larger impact on zooplankton populations than juvenile trout. It was not possible to make any definitive conclusions on how stocked fish size may affect prey resources within lakes.

Trout Stocking and Lake Food Web

Most research on the effects of trout stocking on lake food-webs has occurred in unproductive systems where the introduction of stocked trout negatively impacts native biota. Of the few studies conducted in more productive systems, minimal changes in benthic invertebrate assemblages were observed. Studies addressing trout stocking in productive lakes, or lakes with a history of fish stocking, and the subsequent effects on microcrustacean communities are rare and suggested various but inconsistent effects between studies. The unique features of each waterbody, number and type of fish stocked, overstocking, and intraspecific competition for food resources were some of the variables that likely influence study results.

Trout Stocking and Nutrients

In relatively unproductive lakes, introduced trout fundamentally altered nutrient cycles and stimulated primary production. Stocked trout were found to access and distribute benthic phosphorus sources that were not formally available to pelagic communities of oligotrophic lakes. In more productive lakes trout stocking also influences nutrient cycling. One study found that a stocking moratorium resulted in lower surface water algae biomass, clearer water, and significantly lower total phosphorus (TP) compared to pre-moratorium years. It was hypothesized that lower TP was a result of the increased standing stock of large bodied *Daphnia* due to a reduction in predation pressures. Stocked trout have also been found to excrete significantly more phosphorus than trout in systems with introduced, but self-sustaining trout populations. However, in Canadian boreal lakes there was no significant difference in chlorophyll-a, the ratio of chlorophyll-a to TP, or the ratio of chlorophyll-a to total nitrogen between stocked, unstocked, and fishless lakes.

Assumptions and Hypotheses

Using the literature review and general limnological theory, the following working assumptions and hypotheses were constructed and used throughout this study:

1. Algae blooms of primary concern are those comprised of cyanobacteria, which include some species that produce various cyanotoxins at unpredictable rates.
2. Cyanobacteria blooms are primarily driven by the phosphorus supply (i.e., bottom up control).
3. Cyanobacteria blooms may be stimulated on occasion by the nitrogen supply for some species that can use (fix) atmospheric nitrogen when nitrate and ammonia concentrations are low.
4. Cyanobacteria blooms also may be stimulated in part by climatic factors including high water temperatures and low wind mixing that favor cyanobacteria growth over other types of algae.
5. Toxic cyanobacteria blooms most often occur during the late summer or fall, but can occur at other times of year in some lakes.
6. Cyanobacteria blooms may be reduced by zooplankton grazing (i.e., top down control), primarily by *Daphnia* and other large cladocerans that can consume small to moderately-sized cyanobacteria colonies.
7. Cyanobacteria blooms may be stimulated by preferential grazing of zooplankton on other types of more edible algae.
8. Cladoceran populations primarily increase in response to algae growth as their primary food source, and often reach their maximum abundance in the spring
9. Legal size trout plants most commonly occur in the spring before opening day of fishing season in late April, which is when the lake phytoplankton community is typically dominated by diatoms and small edible algae that stimulate high cladoceran abundance.
10. Cladoceran populations decrease from predation by small fish, which primarily include small warm-water fish and trout up to legal size in Washington lakes, but may also decrease from predation by predatory zooplankton species such as *Chaoborus*.
11. Stocking lakes with small trout may decrease cladoceran populations and stimulate cyanobacteria blooms.
12. Stocking lakes with large trout and other piscivorous fish (e.g., walleye) that prey on small fish may increase cladoceran populations and reduce cyanobacteria blooms.

SUPPLEMENTAL REVIEW

An additional eight papers were reviewed that related to fish stocking and lake food webs. This supplemental review is provided below followed by the paper abstracts. The additional citations are added to the References section.

Fish Stocking and Lake Food Webs

Salmonids, which are not native to the Southern Hemisphere, are now widely distributed throughout Patagonia (Pascual et al. 2009). Introductions of salmonids and other exotic fish species have caused top-down effects in many lakes leading to a severe degradation in the quality of native fisheries (Pascual et al. 2009). Pascual et al. (2009) suggest other top-down effects related to fish introductions may cause decreases in zooplankton populations and enhance phytoplankton blooms. Indeed, a separate study on 18 Patagonia lakes showed clear differences in plankton composition and zooplankton size in lakes with and without stocked fishes (Ressig et al. 2006). Trout were the dominant fish species in four of the five stocked lakes. Stocked lakes had homogenous crustacean plankton communities dominated by rotifers whereas most fishless lakes were dominated by large and medium sized centropagids (e.g., *Parthenina sarsi*) and cladocerans (e.g., *Daphnia* spp.) (Ressig et al. 2006). In all stocked lakes, fish introductions caused changes to the plankton structure that cascaded down to phytoplankton. Cyanobacteria were dominant in lakes where introduced fishes were present (Ressig et al. 2006).

Similar top-down effects were observed in pristine high-altitude Ecuadorian Andes lakes and western Italian Alps lakes (Mouillet et al. 2018, Tiberti et al. 2014). Brook Trout (*Salvelinus fontinalis*) were introduced into Italian Alps lakes causing losses of large bodied zooplankton biomass (Tiberti 2014). However, no significant differences in zooplankton biomass were detected between stocked and fishless lakes because loss of large zooplankton species was offset by increases in small zooplankton species (Tiberti 2014). In shallow Ecuadorian lakes stocked with Rainbow Trout (*Oncorhynchus mykiss*) significant differences were also documented in the taxonomic composition of zooplankton communities when compared to fishless lakes (Mouillet et al. 2018). Although zooplankton contributed only a minor fraction of the trout diet, *Daphnia* were only dominant in two of the seven stocked lakes whereas *Daphnia* dominated the zooplankton community of all fishless lakes (Mouillet et al. 2018). In the two stocked lakes only small bodied *Daphnia* (i.e., *D. ambigua*) were present while fishless lakes were dominated by large bodied *Daphnia* (i.e., *D. pulicaria*, *D. pulex*, and *D. schodleri*) (Mouillet et al. 2018). No significant cascading effects on phytoplankton were discovered although chlorophyll-a was slightly higher in stocked lakes. This could be because the high-altitude lakes are so nutrient poor.

In contrast to the studies described above, large cladoceran taxa dominated the zooplankton community in the historically fishless Diamond Lake in the Cascade Range, Oregon even after rainbow trout were introduced (Eilers et al. 2007). Although some modest changes in water

quality and lake biota were documented after trout were stocked, major changes to the zooplankton community were only documented after the introduction of tui chub (*Gila bicolor*). After the introduction of tui chub, the size of cladocerans decreased and the zooplankton community became dominated by rotifers and smaller bodied crustaceans (Eilers et al. 2007). Major increases in cyanobacteria were also noted after the tui chub introduction.

Abstracts

Bottrell et al. 1976

The problems associated with the measurements of the three main variables used in production estimates – standing crop, individual weights, and development times – are reviewed within the context of attaining absolute comparisons between water bodies. The paper is mainly based on data collected during the International Biological Program.

Christensen and Moore 2007

In Twin Lakes, Washington, illegal introductions of largemouth bass (*Micropterus salmoides*) and golden shiner (*Notemigonus crysoleucas*) are feared to be impacting economically important rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) populations. We evaluated the stomach contents of 69 golden shiner, 146 rainbow trout, 83 brook trout, and 561 largemouth bass during summer stratification in 2004 and 2005, to determine community diet composition and overlap when food resources were partitioned thermally and spatially. Gut content data revealed some diet overlap but also illustrated distinct resource partitioning among all species but not between salmonids. Rainbow and brook trout had similar pelagic based diets of zooplankton and chaoborids, with high estimated diet overlap. Largemouth bass ≥ 300 mm was piscivorous and consumed principally golden shiner, with some consumption of rainbow and brook trout during late spring and early fall. Largemouth bass ≤ 299 mm primarily consumed benthic invertebrates in littoral macrophyte beds. Golden shiner diet contained both littoral and pelagic items, consisting of algae, benthic invertebrates, and zooplankton. Preferential differences in temperature, dissolved oxygen, and habitat as well as species size and ontogeny may all contribute to resource partitioning in the Twin Lakes.

Eilers et al. 2007

Biological responses to a formerly fishless lake in the Cascade Range, Oregon (USA) were assessed through monitoring of recent changes and paleolimnological techniques to assess earlier changes. We used unpublished fisheries reports, sediment cores, and available published and unpublished water quality data to evaluate changes to the lake. Diamond Lake has undergone four periods of fish introductions in the 20th century. Rainbow trout (*Salmo gairdneri*) were annually released from 1910–2006, except for 1949 and 1954. Tui chub (*Gila bicolor*), an omnivorous cyprinid, were introduced in the late 1930s and the late 1980s, presumably as discarded live bait. Diamond Lake was treated with rotenone in 1954 which

successfully eradicated the tui chub. The introductions of trout caused relatively modest changes in water quality and lake biota, whereas the introductions of tui chub caused major increases in cyanobacteria, changes in diatom community composition, reduction in transparency, increases in the proportion of rotifers, major reduction in benthic standing crop, and virtual elimination of amphipods, gastropods, and other large-bodied invertebrates. The unintended biomanipulations demonstrate the importance of an omnivorous cyprinid in promoting a series of biological responses throughout the lake food web.

Hartman and Kunkel 1991

A conceptual behavioral and mechanistic Holling-type model of food selection in *Daphnia pulicaria* is derived from SEM observations with animals feeding on mixtures of spherical-cylindrical diatoms, oblong green algae, and filamentous cyanobacteria, as well as ultrafine particles. The algae used were *Stephanodiscus hantzschii* ($\leq 6 \mu\text{m}$ length), *Monoraphidium setiforme* ($\geq 20 \mu\text{m}$), and *Oscillatoria aghardii* (strands, $\geq 80 \mu\text{m}$). Cell (strand) selection can occur at any or all of three stages: (i) interception from the feeding currents, (ii) collection and channeling to the food groove, and (iii) compaction and transport to the mouth. During each stage, given equal initial cell densities, elongate cells are more likely to escape collection than spherical cells and are more likely to be rejected. In addition, filaments require increased handling time at stages (ii) and (iii) and promote entanglement with limb 5 and the post-abdominal claw. Food is collected primarily with the aid of limbs 3 (and 4), but limbs 1 and 2 also intervene. Neither the leaky sieve hypothesis alone nor any other single-process hypothesis explains the observations on examined in corpore positions, morphology, and derived movements of the feeding limbs. Attachment and mucus appear to be important for the ingestion of bacteria and ultrafine particles. The model is consistent with many experimental results of differential feeding by *Daphnia pulicaria* on mixtures of variously shaped algae and other observations on *Daphnia* feeding behavior. The paradigm of invariant, nonselective feeding by *Daphnia* is rejected.

Mouillet et al. 2018

High altitude waters in the Ecuadorian Andes are devoid of native fish, but rainbow trout (*Oncorhynchus mykiss*) have been introduced widely. We surveyed 14 small and shallow, high altitude (3800 – 4300 m a.s.l.) lakes in Ecuador; seven lakes without fish, and seven with introduced rainbow trout. The main purpose of this study was to explore the effects of introduced rainbow trout on biomass, abundance and composition of pelagic (phyto- and zooplankton) and benthic (algae and macroinvertebrate) communities in lakes where fish are naturally absent. As expected based on studies from temperate zone high alpine lakes, there were considerably (although non-significant) lower mean total zooplankton biomass (13 versus 105 $\mu\text{g/L}$ DW) and higher mean phytoplankton biomass (2.26 versus 1.56 $\mu\text{g/L}$ Chl-a) in lakes with than in lakes without fish, and significant differences in the taxonomic composition of the zoo- and phytoplankton community were observed between the two groups of lakes. While the genus *Daphnia* dominated in almost all of the fishless lakes (88 compared to 4 $\mu\text{g/L}$ DW, respectively), other genera of smaller-sized cladocera (or calanoid copepods) dominated in the

fish lakes. Fish caught in gill nets ranged from 8.6 to 24.5 cm (fork length) and the weight was 6.0 to 199.5 g WW. Analyses of stomach content showed that all size classes had consumed considerable proportions of macrophyte material and filamentous algae. Of the consumed animal prey, benthic macroinvertebrates were by far the main food source, even for the smallest size classes (82 –100 percent by volume for individual fish, overall mean 99 percent). In contrast, zooplankton contributed only 0 –18 percent of consumed animal prey (overall mean 1 percent). In conclusion, our study showed that the introduction of an alien predator such as the rainbow trout to naturally fishless equatorial high Andean lakes had effects on the pelagic part of lake food webs, mainly on the cladoceran community.

Pascual et al. 2009

Introduced species have become established throughout large areas of the world, causing millions of dollars in damages. The introduction of such pest species is universally condemned, and science and management efforts are geared toward eradication, containment, or prevention of future infestations. Meanwhile, other organisms are actively traded around the world for consumption, as well as recreational and aesthetic purposes, providing examples of the conflict between human development and conservation. When dealing with such species, are there ways to balance the competing goals of economic production and protection of nature? How can science help to identify suitable compromises? We address these questions by analyzing three case studies dealing with exotic salmonids in Patagonia: trout aquaculture in shallow, fishless lakes; trout recreational fisheries; and marine net-pen salmon aquaculture. We propose that three interrelated properties of these case studies (scale, connectivity, and incentives for conservation) determine our ability to identify and promote situations that balance production and the integrity of nature.

Ressig et al. 2006

Patagonia fishless ponds have been stocked with fishes for recreational purposes since early in the 20th century. We carried out a summer plankton sampling in 18 Patagonia lakes; 12 fishless, 5 with introduced fishes and 1 with endemic fish fauna. The lakes are situated on a latitudinal gradient from 39° to 49°S. Zooplankton and phytoplankton composition, phytoplankton relative abundance, and zooplankton body size and mouthpart morphology were analyzed. Results showed differences between lakes with and without fishes; in the presence of fish zooplankton size spectrum tended to be narrower because of the disappearance of *Daphnia* and large centropagid copepods. Zooplankton composition changed: centropagid species richness decreased and rotifers dominated. Contrarily, in fishless lakes 3 or 4 centropagid species, differing markedly in body size and exploiting different food niches, were observed co-occurring. These changes in zooplankton seemed to cascade down to phytoplankton. Fish introduction increased the phytoplankton similarity in lakes even belonging to different basins in a latitudinal gradient. Indeed, cyanobacteria dominated only in lakes with introduced fishes. Probably the elimination of *Daphnia* favored cyanobacteria proliferation due to nutrient rebalance. As a consequence, water quality decreases and the value of sport fisheries is reduced.

Fish introduction in Patagonia is a practice that should be re-evaluated by governments and NGOs due to its potentially negative impact on lakes and local economies.

Tiberti et al. 2014

Fish introduction is a major threat to alpine lake biota leading to the loss of native species and to the degeneration of natural food-webs. This study provides an extensive investigation on the impact of the introduced fish *Salvelinus fontinalis* on the native communities of alpine lakes in the Gran Paradiso National Park. We compared the macroinvertebrate and zooplankton communities of six stocked and nine fishless lakes with a repeated sampling approach during the summers 2006–2009. The impact of fish presence on alpine lake fauna is often mediated by the strong seasonality governing these ecosystems, and it dramatically affects the faunal assemblage of littoral macroinvertebrates and the size, structure, and composition of the pelagic zooplankton community with a strong selective predation of the more visible taxa. Direct ecological impacts include a decrease or extinction of non-burrower macroinvertebrates and of large zooplankton species, while small zooplankton species and burrower macroinvertebrates were indirectly advantaged by fish presence. Due to the existence of a compensation between rotifers and crustaceans, fish presence does not affect total zooplankton biomass and diversity even if fish are a factor of ecological exclusion for large crustaceans. These compensatory mechanisms are a key process surrounding the impact of introduced fish in alpine lakes.

LIBERTY LAKE DATA ANALYSIS

METHODS

Data Availability

A data analysis plan is presented in Appendix B that describes what data were available and the initial plan for analyzing the Liberty Lake data. In this plan, the period of record, sampling locations, sampling frequency, and data gaps are identified for lake water quality (field and laboratory parameters), phytoplankton, zooplankton, cyanotoxin, and fish stocking data for Liberty Lake. Data availability is summarized below in Table 1. Nutrients include total phosphorus, orthophosphate, total nitrogen, nitrate+nitrite nitrogen, and ammonia nitrogen. Field parameters include Secchi depth, temperature, dissolved oxygen, pH, and conductivity. Cyanotoxins include microcystin and anatoxin-a. Fish stocking species primarily include rainbow trout, brown trout, and walleye.

Nutrients and phytoplankton samples were collected from three depths (near surface, mid-depth, and near bottom) at two lake stations named Northwest (NW) and Southeast (SE) (Figures 1 and 2). The NW station is located at the deeper portion of the lake and therefore the data collected at this site are assumed to be the best representation of the lake. Field parameter and *in-situ* chlorophyll-a data were collected in profiles at 1-meter increments at both stations. Secchi depth was also measured at each station during sampling events. Zooplankton were sampled in one vertical tow from each station. Cyanotoxin samples were collected from surface scums when they were observed at beaches. The Liberty Lake watershed is presented in Figure 1 and the monitoring station locations are shown on a bathymetric map in Figure 2.

Table 1. Liberty Lake Data Availability.				
Parameter	Stations	Depths	Frequency	Period
Precipitation	GEG Airport	NA	Continuous	1976-2018
Nutrients	NW, SE	Surface/Mid/Bottom	1-2/month	1977-2018
Field parameters	NW, SE	1-Meter Profile	1-2/month	1983-2018
Secchi Depth	NW, SE	NA	1-2/month	1983/2018
<i>In-Situ</i> Chlorophyll-a	NW, SE	1-Meter Profile	1-2/month	2013-2018
Phytoplankton biovolume	NW, SE	Surface/Mid/Bottom	1-2/month	2000-2018
Zooplankton abundance	NW, SE	Vertical tow	1-2/month	2000-2018
Cyanotoxins	Beaches	Shore scum	2-7/year	2008-2016
Fish stocking	Lake	NA	1-7/year	1967-2018

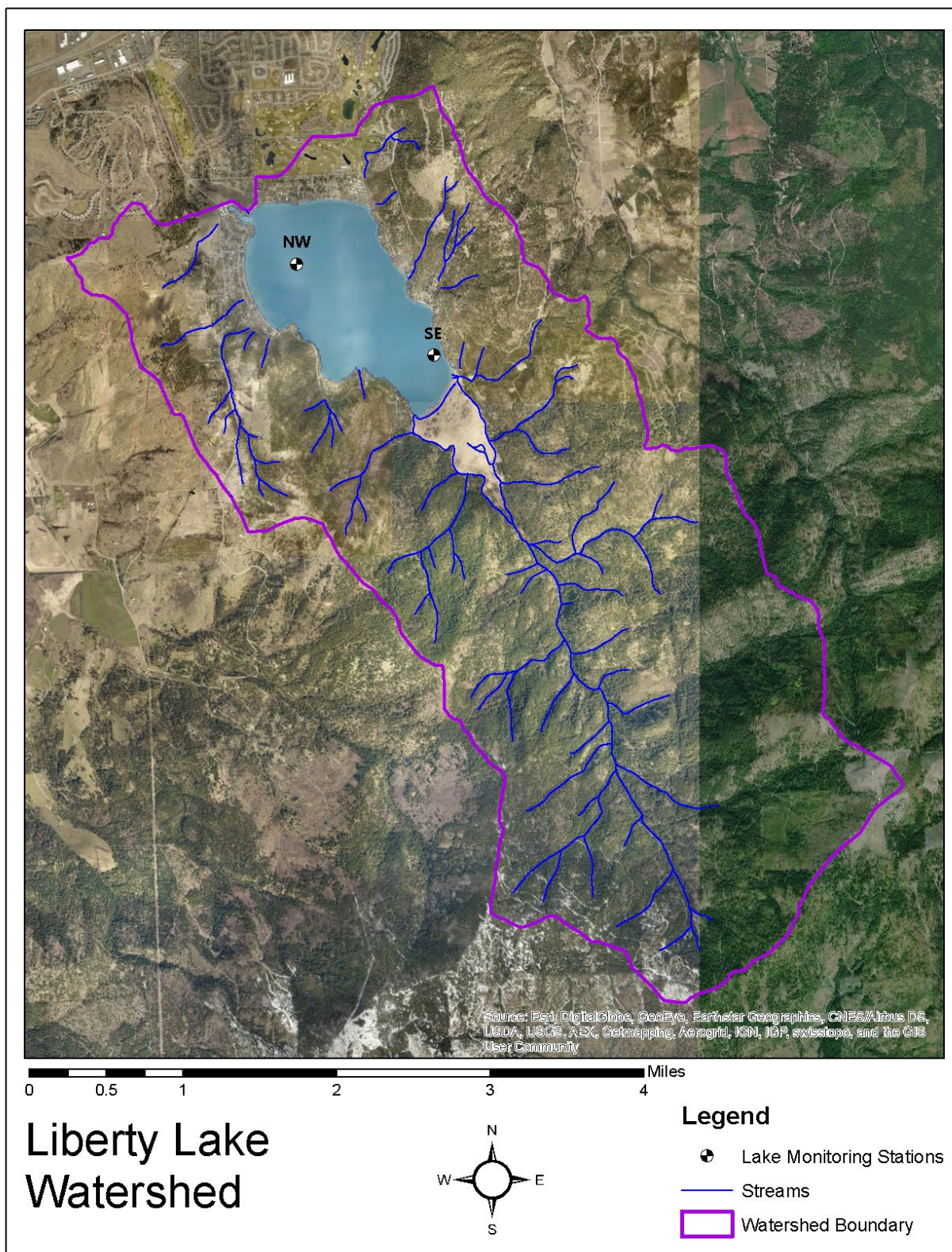


Figure 1. Liberty Lake Location and Watershed Map.

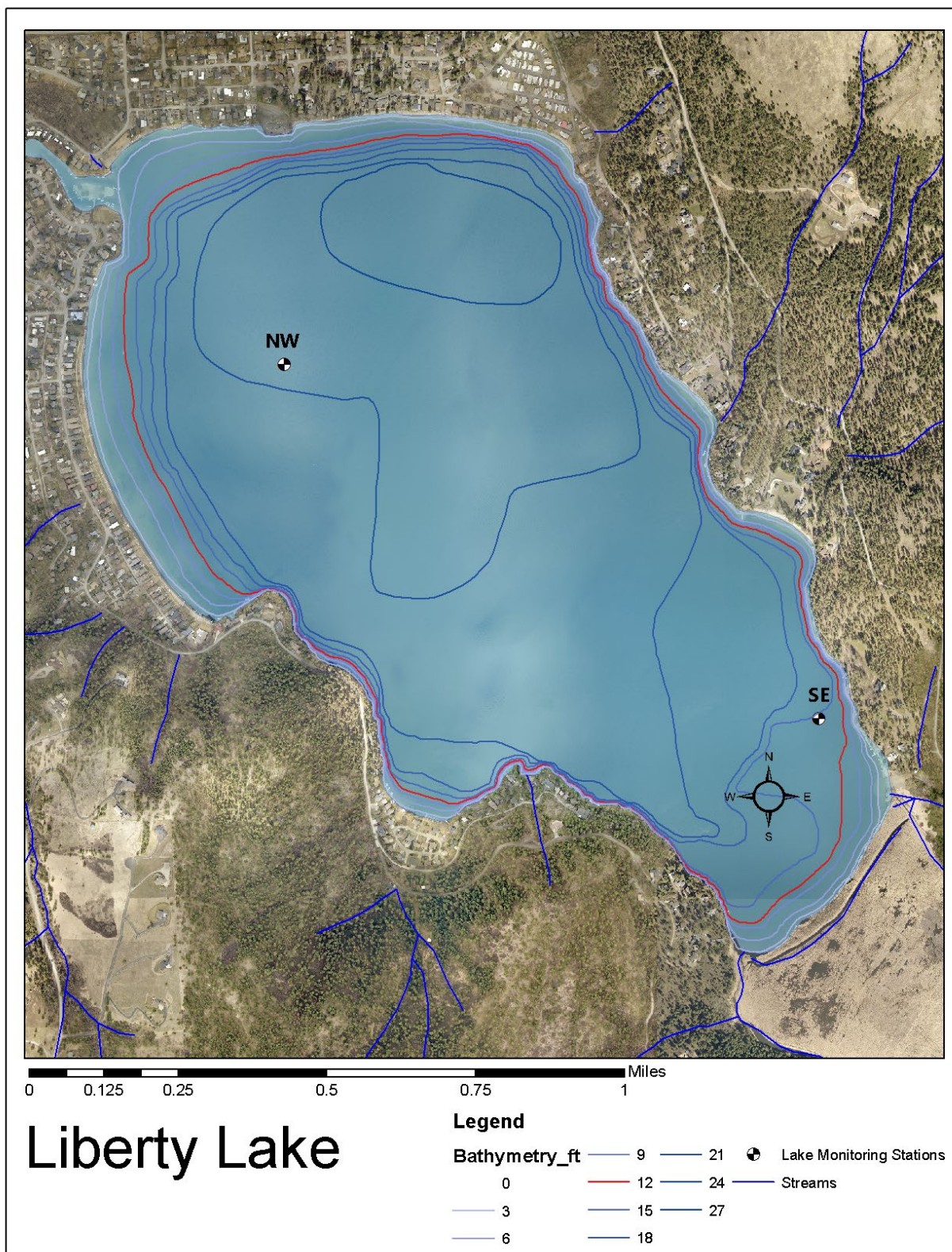


Figure 2. Liberty Lake Water Quality Monitoring Stations (NW and SE).

Water quality data (nutrients and field parameters) were available from April through October in all years for all field parameters since 1983, except for some gaps for total nitrogen and larger gaps for chlorophyll-a. Total monthly precipitation data were compiled from the Spokane International Airport (GEG) since 1976. Phytoplankton data as cell number and biovolume for eight groups (phyla) have been collected consistently since 2000 at the three depths and two lake stations. Zooplankton data have been collected at both stations consistently since 1983 and were compiled for five groups (classes). Cyanotoxin data were only available for a few shore scum samples collected in each of 4 years (2008, 2009, 2015, and 2016).

Fish stocking data were available and complete since 1967. These data include the numbers and biomass (pounds) of each stocked species and size (fry or legal-size) of fish. Most of the fish were planted in the lake during spring season (April through June) and many were also planted in the late summer/fall season (August through October). Fish stocking data show that Liberty Lake has been stocked by a wide variety of species and size classes throughout the years, with fry of all species dominating the numbers stocked on different years, while the total biomass stocked is typically dominated by legal-size rainbow trout followed by legal-size brown trout (see Appendix B). On occasion, kokanee, brook, and tiger trout have been stocked. Additionally, channel catfish and sauger (similar to walleye) were stocked during the study period.

Database Development

Although data extended back to 1977 for some parameters, only years with a complete data set for all parameters are considered in the present study that includes each year from 2000 through 2018 except 2005. Mean values for meteorology, water quality, phytoplankton, zooplankton, and fish stocking parameters were calculated for each year from 2000-2018 (except 2005) for the following four periods:

- Spring (May-June)
- Summer (July-August)
- Fall (Sept-Oct)
- Year (May-Oct)

Beside zooplankton and fish stocking data, for each period mean values were calculated for three depths (top, mid, and bot) at each of the two stations, resulting in 6 sampling sites for each period. Zooplankton data were only available from vertical tow samples so mean zooplankton values were associated to each depth at the sampled station. Fish stocking data, representing the entire lake, were applied to each station and depth. Fish stocking counts and biomass were totaled for each season by two groups: all trout species (coded as TK) and walleye and warmwater species (coded WW); for three size classes: all sizes, fry, and legal; and for two different units: mass and number.

The resulting database is a robust and holistic dataset with a variety of water quality parameters (nutrients and field parameters), cell biovolume concentration for each phytoplankton species and phyla groups, concentration of zooplankton species and class groups, and numbers and mass of each fish group. This resulted in 78 metrics that include 1 meteorological metric, 11 water quality metrics, 18 phytoplankton metrics, 2 cyanotoxin metrics, 34 zooplankton metrics, and 12 fish stocking metrics shown in Table 2. Each period, station, and depth have an associated set of these 78 metrics that is partially unique to that specific site.

Data Analysis

Temporal and Spatial Patterns

Data for each depth (top, mid, and bot) at each station (NW and SE) were used to create six time series plots that include annual means/totals of five key parameters: total algae biovolume ($\mu\text{m}^3/\text{mL}$), total cyanobacteria biovolume ($\mu\text{m}^3/\text{mL}$), total *Daphnia* abundance (number/ m^3), total phosphorus concentration (mg/L), and total trout stocking mass (lbs). Due to varying units of measurement among these parameters, the plots show normalized data based on their maximum value at that specific station-depth. These plots were reviewed by visual inspection to identify any initial trends or relationships in the parameters prior to conducting statistical analyses.

Principal Component Analysis

Principal component analysis (PCA) is a technique for simplifying a data set so that broad patterns may be more readily detected. In principal component analysis, the data are transformed to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (referred to as the first principal component), the second greatest variance on the second coordinate, and so on (Ludwig and Reynolds 1988; StatSoft 1994). Principal component analysis can be used for dimensionality reduction in a data set while retaining those characteristics of the data set that contribute most to its variance, by keeping lower order principal components and ignoring higher order ones. Such low-order components often contain the “most important” aspects of the data.

PCA uses a matrix as an input with rows referred to as “objects” and columns termed “variables”. In this application, the variables are denoted as the parameters with the objects being the time series of data collected for each of the four time periods (spring, summer, fall, and year) and all seasons combined from 2000-2018. Prior to analysis, the data for each variable were standardized using the following formula:

Standard score = (raw score – mean)/standard deviation

The *prcomp()* function for the R statistical software package was then used to perform PCA analyses on a subset of the 78 metrics for the top depth station for each season, the entire year, and exclusively for full data set years, respectively. The function only works for complete data sets so years or seasons with data gaps were dropped from the analysis. Table 3 presents the 33 metrics (variables) used for the PCA that were key parameters with complete data sets.

Table 2. Liberty Lake Data Analysis Variables.

Parameter	Group	Code	Parameter	Group	Code
Total Precipitation	Precip	Pre	Total Zooplankton	Zoo	ZG
Temperature	WQ	T	Alona	Zoo species	ZS1
Temperature Diff. (S-B)	WQ	TD	Bosmina	Zoo species	ZS2
Dissolved Oxygen	WQ	DO	Ceriodaphnia	Zoo species	ZS3
DO Minimum	WQ	DOM	Chydorus	Zoo species	ZS4
Chlorophyll-a	WQ	C	D. ambigua	Zoo species	ZS5
Secchi Depth	WQ	S	D. dubia	Zoo species	ZS6
Nitrate+Nitrite,	WQ	NN	D. galeata mendotae	Zoo species	ZS7
Ammonia	WQ	NH3	D. laevis	Zoo species	ZS8
Total Nitrogen	WQ	TN	D. parvula	Zoo species	ZS9
Orthophosphate	WQ	OP	D. pulex	Zoo species	ZS10
Total Phosphorus	WQ	TP	D. pulicaria	Zoo species	ZS11
Total Phytoplankton	Phyto	PG	D. retrocurva	Zoo species	ZS12
Total Cyanobacteria	Phyto	PGCS	D. rosea	Zoo species	ZS13
Total Bacillariophyta	Phyto	PG1	D. schodleri	Zoo species	ZS14
Total Chlorophyta	Phyto	PG2	Daphnia thorata	Zoo species	ZS15
Total Chrysophyta	Phyto	PG3	Diacyclops	Zoo species	ZS16
Total Cyanophyta	Phyto	PG4	Diaphanosoma	Zoo species	ZS17
Total Euglenophyta	Phyto	PG5	Diaptomus	Zoo species	ZS18
Total Pyrrhophyta	Phyto	PG6	Epischura	Zoo species	ZS19
Anabaena	Phyto_Species	PS1	Holopedium	Zoo species	ZS20
Anacystis	Phyto_Species	PS2	Juvenile Daphnia	Zoo species	ZS21
Aphanizomenon	Phyto_Species	PS3	Leptodora	Zoo species	ZS22
Aphanocapsa	Phyto_Species	PS4	Mesocyclops	Zoo species	ZS23
Coelosphaerium	Phyto_Species	PS5	Nauplii	Zoo species	ZS24
Gleotrichia	Phyto_Species	PS6	Ostracoda	Zoo species	ZS25
Gomphosphaeria	Phyto_Species	PS7	Sida crystallina	Zoo species	ZS26
Merismopedia	Phyto_Species	PS8	Trout Mass - All Sizes	Fish	FTMA
Microcystis	Phyto_Species	PS9	Trout Mass - Fry	Fish	FTMF
Oscillatoria	Phyto_Species	PS10	Trout Mass - Legal	Fish	FTML
Anatoxin-a	Cyanotoxins	CA	Trout Number - All	Fish	FTNA
Microcystin	Cyanotoxins	CM	Trout Number - Fry	Fish	FTNF
Total Daphnia	Zoo	ZGDS	Trout Number - Legal	Fish	FTNL
Total Chaoboridae	Zoo	ZG1	Warmwater Mass - All	Fish	FWMA
Total Cladocera	Zoo	ZG2	Warmwater Mass - Fry	Fish	FWMF
Total Copepoda	Zoo	ZG3	Warmwater Mass - Legal	Fish	FWML
Total Daphnia	Zoo	ZG4	Warmwater Mass - All	Fish	FWNA
Total Nauplii	Zoo	ZG5	Warmwater Mass - Fry	Fish	FWNF
Total Rotifera	Zoo	ZG6	Warmwater Mass - Legal	Fish	FWNL

Table 3. Liberty Lake Principal Component Analysis Variables.			
Parameter	Group	Code	Unit
Total Precipitation	Precip	Pre	in
Temperature	WQ	T	°C
Temperature Difference (S-B)	WQ	TD	°C
Dissolved Oxygen	WQ	DO	mg/L
Dissolved Oxygen Minimum	WQ	DOM	mg/l
Secchi Depth	WQ	S	m
Nitrate+Nitrite,	WQ	NN	mg/L
Ammonia (NH3)	WQ	NH3	mg/L
Orthophosphate	WQ	OP	mg/L
Total Phosphorus	WQ	TP	mg/L
Total Phytoplankton	Phyto	PG	µm ³ /mL
Total Cyanobacteria	Phyto	PGCS	µm ³ /mL
Total Bacillariophyta	Phyto	PG1	µm ³ /mL
Total Chlorophyta	Phyto	PG2	µm ³ /mL
Total Cryophyte	Phyto	PG3	µm ³ /mL
Total Pyrrophyta	Phyto	PG6	µm ³ /mL
Total Zooplankton	Zoo	ZG	zoo/m ³
Total Daphnia	Zoo	ZGDS	zoo/m ³
Total Cladocera	Zoo	ZG2	zoo/m ³
Total Copepoda	Zoo	ZG3	zoo/m ³
Total Rotifera	Zoo	ZG6	zoo/m ³
Trout Mass - All Sizes	Fish	FTMA	lbs
Trout Mass - Fry	Fish	FTMF	lbs
Trout Mass - Legal	Fish	FTML	lbs
Trout Number- All	Fish	FTNA	Count
Trout Number - Fry	Fish	FTNF	Count
Trout Number - Legal	Fish	FTNL	Count
Warmwater Mass - All	Fish	FWMA	lbs
Warmwater Mass - Fry	Fish	FWMF	lbs
Warmwater Mass - Legal	Fish	FWML	lbs
Warmwater Mass - All	Fish	FWNA	Count
Warmwater Mass - Fry	Fish	FWNF	Count
Warmwater Mass - Legal	Fish	FWNL	Count

In each case the first and second principal components were extracted with their associated eigenvalues. An eigenvalue is a measure of the variance accounted for by each principal component. This information was subsequently used to generate a standard PCA plot for the seasons and years, a circle of correlation for the input parameters, and a parallel axis plot that indicates the variability captured by a given principle component. These plots are interpreted based on the grouping of input parameters or years as well as their distance from the origin. If

parameters are grouped, they are positively correlated. Likewise, if parameters are on opposite sides of the plot (in x or y), they are negatively correlated. The distance from the origin is representative of their influence on the given principle component with more distance being more important.

Correlation Analysis

Prior to conducting multiple regression analyses (see description below), potential independent variables were evaluated for multicollinearity using correlation analyses. Variables were then selectively excluded from the multiple regression analyses if they were found to be significantly correlated with other variables. Initially, 78 parameters (Table 2) were reduced to 22 key parameters that theoretically had no codependence on each other. To confirm this, a Kendall's Tau correlation coefficient was calculated for all combinations of the 78 variables. This analysis was performed using *ggcorr()* function from the "GGally" package for the R statistical software package with the method set to "kendall". Parameters that were shown to be significantly correlated with $\alpha = 0.1$ (Helsel and Hirsch 2002) were not analyzed together in the multiple regression analysis. Excluding those identified by theory, only two statistically significant correlations were found:

- Cyanobacteria – Microcystis
- Total Zooplankton – Nauplii

Multiple Regression Analysis

Multiple regression analysis is a statistical process used to estimate dependent variables based on at least two independent variables. Using the 22 key parameters identified in the correlation analysis, 9 key parameters were selected to be analyzed as the dependent variables in the multiple regression analysis. The dependent variables and associated independent variables are shown in Table 4. For each dependent variable, all 6 station-depths are considered. The analysis was conducted using the *lm()* function in the R statistical software package.

The results from each of these analyses were summarized using plots showing the beta coefficients from the regression models for each independent variable and the associated p-value to indicate the significance. For a parameter to be considered significant, $p\text{-value} < \alpha = 0.1$ (Helsel and Hirsch 2002). A standardized beta coefficient compares the strength of the effect of each individual independent variable to the dependent variable. The higher the absolute value of the beta coefficient, the stronger the effect. In other words, the beta coefficient (β) indicates the sensitivity of the dependent variable to change with respect to the independent variable. For example, a beta of -0.9 has a stronger effect than a beta of +0.8.

Table 4. Dependent Variables (Bold) with the Associated Independent Variable								
PG	PGCS	PS1	PS3	PS9	ZG	ZGDS	ZG3	ZS18
FTMA	FTMA	FTMA	FTMA	FTMA	FTMA	FTMA	FTMA	FTMA
FWMA	FWMA	FWMA	FWMA	FWMA	FWMA	FWMA	FWMA	FWMA
TP	TP	TP	TP	TP	TP	TP	TP	TP
OP	OP	OP	OP	OP	OP	OP	OP	OP
NN	NN	NN	NN	NN	NN	NN	NN	NN
T	T	T	T	T	T	T	T	T
TD	TD	TD	TD	TD	TD	TD	TD	TD
ZG	ZG	ZG	ZG	ZG	ZS22	ZS22	ZS22	ZS22
ZGDS	ZGDS	ZGDS	ZGDS	ZGDS	PG	PG	PG	PG
ZG3	ZG3	ZG3	ZG3	ZG3	PGCS	PGCS	PGCS	PGCS
ZG5	ZG5	ZG5	ZG5	ZG5	PG1	PG1	PG1	PG1
ZG6	ZG6	ZG6	ZG6	ZG6	PG2	PG2	PG2	PG2
ZS18	ZS18	ZS18	ZS18	ZS18	PG3	PG3	PG3	PG3
					PS1	PS1	PS1	PS1
					PS3	PS3	PS3	PS3
					PS9	PS9	PS9	PS9

See Table 2 for code definitions.

RESULTS

The figures discussed in the following sections are all provided in Appendix C. Prior to the discussion of these results there are a few notable considerations.

- This study is primarily focused on the impacts of stocked trout on cyanobacteria and recognizes other parameters influence cyanobacteria populations that vary seasonally and annually. There are several factors that may affect cyanobacteria populations but are not captured in the available data. These include but are not limited to stratification, hypolimnetic anoxia and subsequent internal nutrient loading, external nutrient loading, and anthropogenic alterations (e.g. invasive species introduction, stormwater, chemical treatment). The degree of thermal stratification was addressed simply by taking the difference in surface and bottom water temperatures. The degree of hypolimnetic anoxia was addressed simply by evaluating the minimum dissolved oxygen concentration for each station-depth data set. The degree of stormwater input was addressed simply by evaluating total rainfall. Treatments of Liberty Lake with aluminum sulfate for phosphorus inactivation or with herbicides for Eurasian watermilfoil control were not factored in the analysis.
- The *Liberty Lake Algae Control Plan* (Tetra Tech 2018) identified potential causes of the large cyanobacteria blooms that occurred in 2015 and 2016. It was found that these algae blooms were due to significant stratification or high values of RTRM (relative thermal resistance to mixing). During these years the system was also exposed to low

wind speeds, inflow, and outflow. These conditions increase algal populations, specifically cyanobacteria which thrive in stagnant conditions due to their buoyant characteristics. Internal loading was also found to be a significant source of nutrients in Liberty Lake. Therefore, the complex lake hydrodynamics and nutrient cycling may have bottom-up control of cyanobacteria in Liberty Lake that was not entirely captured in the parameters evaluated for the present study.

- The values for stocked fish may represent only a small portion of the fish populations in Liberty Lake. Stock carryover from previous years and reproducing warmwater fish populations in Liberty Lake are not accounted for in the present study. Similarly, the catch and mortality rate of stocked fish are not considered in the present study.

Temporal and Spatial Patterns

Figures for each of the six station-depths showing times series of annual averages for total algae biovolume, total cyanobacteria biovolume, total Daphnia species numbers, total phosphorus, and mass of stocked trout are presented in Appendix C. The results from this section provide a cursory view of potential patterns to support the statistical analyses in the following sections.

Figure 3 presents an example time series plot for the top depth of the NW station. Here, trout stock mass (red line) reached a maximum value (100 percent normalized value) of approximately 10,000 pounds (lbs) in 2007 and a minimum value (20 percent normalized value) of approximately 2,000 pounds in 2014. Total Daphnia (orange line) showed similar trends for both stations with the most significant peak occurring in 2015 and 2016 when the toxic cyanobacteria blooms were observed, and with lowest value in 2008 and 2009 following the highest trout stock mass in 2007.

Cyanobacteria (blue) were present in the lake every year during the study period but at highly variable concentrations with respect to the station and depth. Throughout the water column at both stations there were relatively high concentrations during 2000-2001 and the toxic bloom years of 2015-2016. There was also a significant peak at the middle and bottom depths of the NW station in 2003 and 2004, respectively. At the bottom depth of the SE station in 2003, the cyanobacteria biovolume was an order of magnitude larger than other observed concentrations, which appears to be an outlier. Total algae (green line) biovolume appears to follow the cyanobacteria trends but with higher concentrations as it includes additional species. As these trends appear to be similar at the top and middle stations, this suggests that cyanobacteria and the other algal species compete for the same resources and nutrients at these depths.

Total phosphorus (yellow line) demonstrated a few significant fluctuations with local maxima and minima varying between stations and depths. At both stations there were higher phosphorus concentrations collected at the deepest sampling depth, which is indicative of internal loading. This pattern only stands if samples were collected during stratified and hypoxic conditions, which supports the findings of the Liberty Lake Algae Control Plan (Tetra Tech 2018).

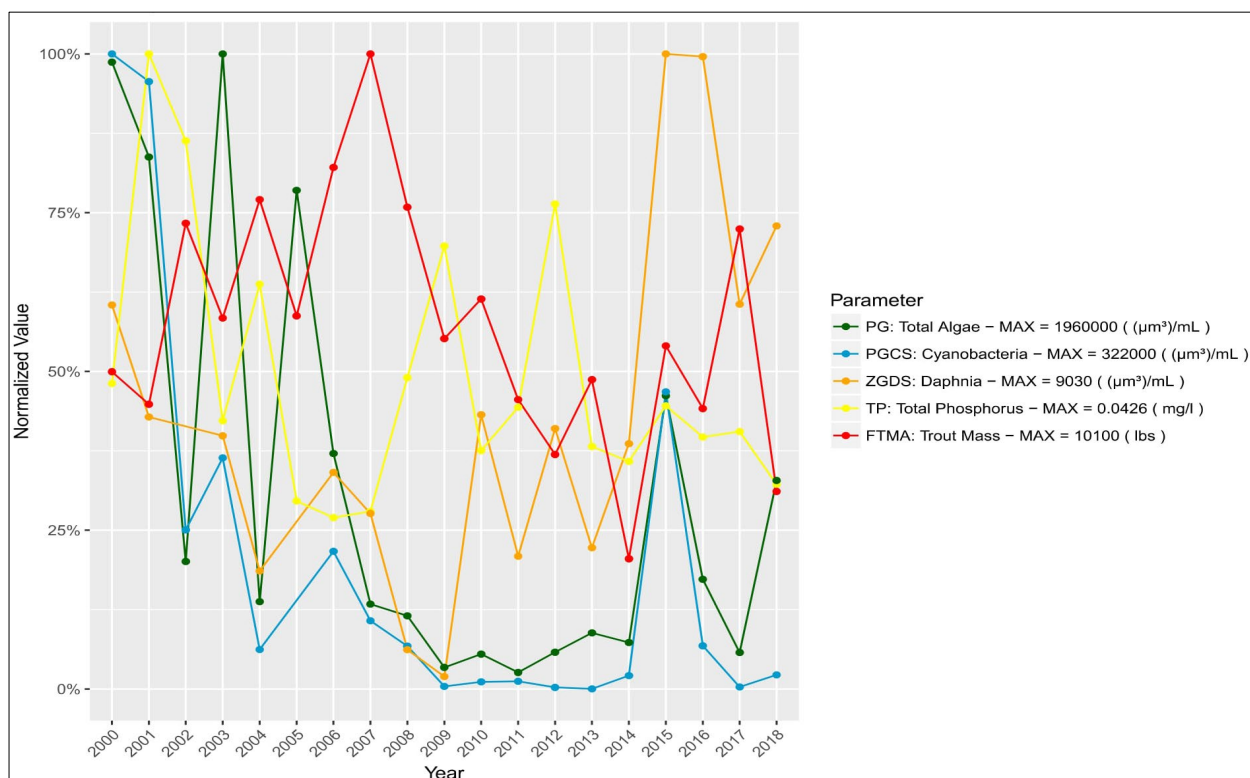


Figure 3. Liberty Lake Normalized Annual Values for Station NW Top Depth, 2000-2018.

Two potential relationships between parameters were visually identified in the time series plots. The first is the negative relationship between total phosphorus (TP) and Daphnia. Throughout the plots it was observed that when TP concentrations were high, Daphnia populations were low and vice versa. This is noticeable from the large differences occurring in 2004, 2009, 2015, and 2016. Second, at the surface there also appears to be a positive relationship between the large increases in Daphnia and cyanobacteria in 2015. This observation may suggest that high Daphnia populations may be associated with the toxic blooms in 2015-2016 in addition to the high internal phosphorus loading. However, these patterns are only speculative from these plots and are evaluated further using statistics of all the available data in following sections.

Principle Component Analysis

Using the variables described in Table 3, PCA was conducted on the Liberty Lake data for all data, the full monitoring year, and each of the three monitoring seasons in 2000-2018 (see Appendix C). Figure 4 presents results from PCA for the full years by displaying the parameters in a correlation circle and including a temporal plot of monitoring years. These plots used the entire data set except 2005 was excluded because it was missing zooplankton data.

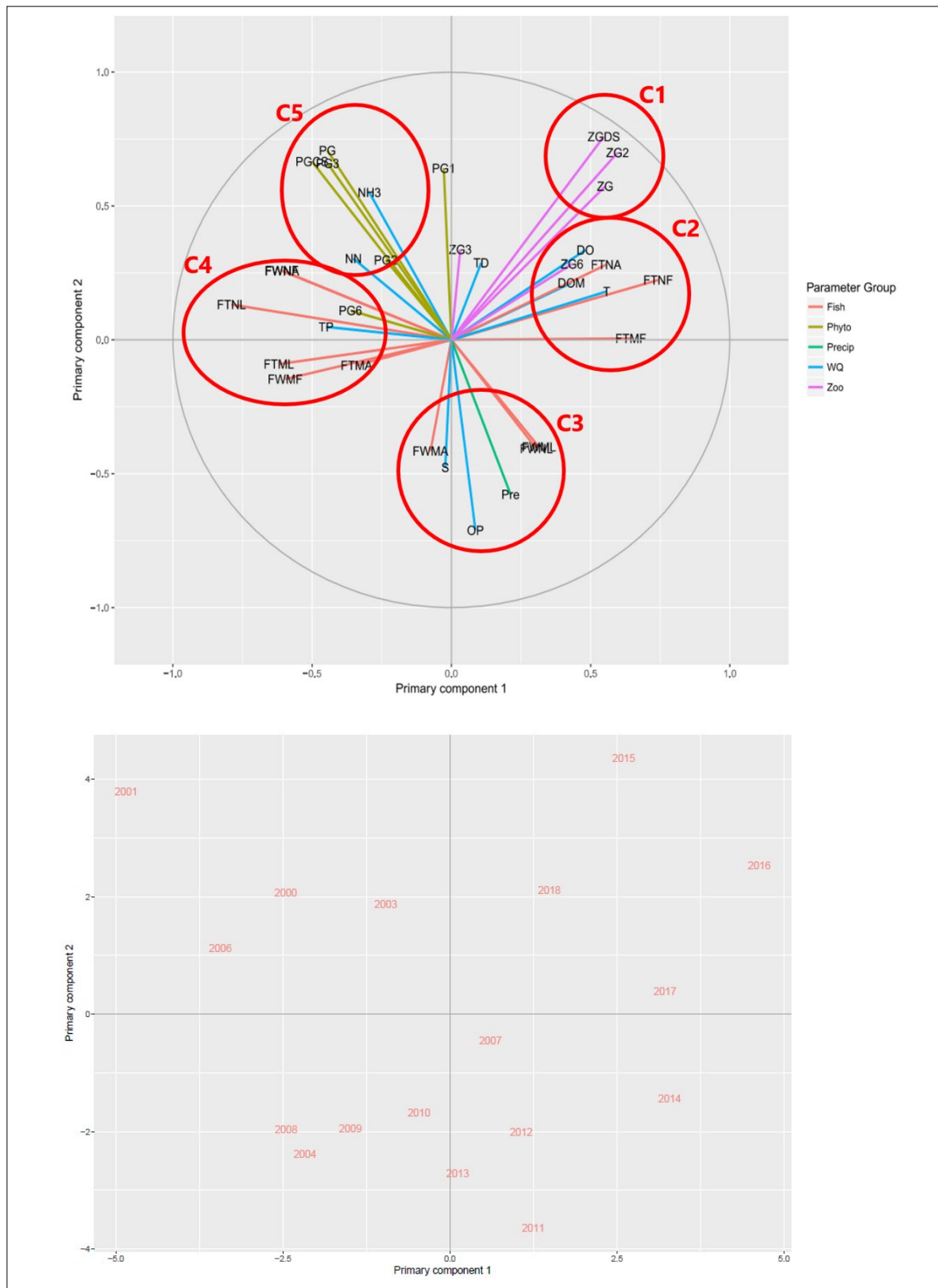


Figure 4. PCA Parameter and Annual Plots of Full Year Data.

The correlation circle shows five clusters of parameters that are either from the same parameter group or intermixed among parameter groups. The following five clusters are named by the most significant parameters followed by specific parameter names and codes in each cluster shown in Figure 4:

- C1 - Zooplankton: total Daphnia species (ZGDS), total Cladocera (ZG2), total zooplankton (ZG)
- C2 – Physical Conditions and Trout Fry: dissolved oxygen (DO), DO minimum (DOM), temperature (T), trout number - all (FTNA), trout number – fry (FTNF), trout mass – fry (FTMF), Daphnia dubia (ZG6)
- C3 – Orthophosphate, Rain, and Secchi Depth: orthophosphate (OP), total precipitation (Pre), Secchi depth (S), warmwater mass – all (FWMA), warmwater number – legal (FWNL), warmwater mass – legal (FWML)
- C4 - Legal Trout: trout number - legal (FTNL), trout mass – legal (FTML), trout mass – all (FTMA), warmwater mass – fry (FWMF), warmwater mass – number (FWNA), warmwater number – fry (FWNF), total phosphorus (TP), total Pyrrhophyta (PG6)
- C5 - Phytoplankton: total phytoplankton (PG), total cyanobacteria (PGCS), total Chlorophyta (PG2), total Chrysophyta (PG3), nitrate+nitrite (NN), ammonia (NH3)

Each cluster in Figure 4 is referred to its designated name in the analysis discussion (e.g. 'legal trout' refers to C4 in Figure 4).

Within each cluster there are notable relationships to consider. In C3, the most significant parameter is orthophosphate (OP) followed by precipitation and Secchi depth. Being grouped together the positive correlation amongst these parameters suggests that more runoff from precipitation is a considerable source of OP but also yields higher water clarity. However, C3 is opposite of high phytoplankton concentrations in C5. This suggests that OP and water clarity are high when phytoplankton are low because phytoplankton uptake OP and reduce water clarity. In C4, total phosphorus was correlated with total trout mass and legal-size trout number. This suggests that substantial phosphorus may be released from stocked trout, as mentioned in the literature review. In C5, nitrate+nitrite and ammonia are all positively correlated with cyanobacteria concentrations, which suggests that inorganic nitrogen may favor cyanobacteria and they do not fix much atmospheric nitrogen because inorganic nitrogen requires less energy to consume, and it was abundant during blooms.

The PCA parameter plot shows the most variability for primary component 1 (x-axis) which was found to explain population interactions between trophic levels. On the x-axis, C1 and C2 are negatively correlated with C4 and C5. These results support a standard trophic cascade pattern. As legal trout stocking (C4) increases, zooplankton (C1) decreases, and phytoplankton (C5) increases. Trout fry (C2) do not have a negative correlation with zooplankton on this axis, which agrees with the findings from the literature review that adult trout have larger impacts on

zooplankton communities than juveniles. Analyzing variability across primary component 2 (y-axis) shows the dependencies and negative correlation of phytoplankton (C5) and zooplankton (C1) to available nutrients (C3). Trout and physical conditions (C2 and C4) do not have a significant amount of variability along the y-axis.

The seasonal PCA plots in Appendix C show how parameter orientations and similarities vary by season. For example, total cyanobacteria (PGCS) are most related to total zooplankton (ZG) and Daphnia (ZGDS) in the spring and summer, but not in the fall when they are most related to nitrate+nitrite. Also, total cyanobacteria (PGCS) are consistently most opposite orthophosphate (OP) in all three seasons.

The PCA year plot at the bottom of Figure 4 shows groupings of similar years. One interesting pattern is that the most recent four years (2015-2018) are the only years in the upper right quadrant where zooplankton are also located in the parameter plot. This grouping suggests that the most recent years are different than previous years and zooplankton parameters are particularly important in recent years.

Key relationships identified by PCA include:

- Liberty Lake exhibits standard trophic interactions with increased trout stocking decreasing zooplankton and increasing phytoplankton.
- Total phosphorus increases with trout stocking, possibly due to phosphorus in trout excretion or in increasing phytoplankton biovolume.
- Stocking of legal-sized trout reduce zooplankton more than stocking of trout fry.
- Total phytoplankton and cyanobacteria concentrations are closely related with each other and with inorganic nitrogen.
- Orthophosphate concentrations are highest while inorganic nitrogen and phytoplankton concentrations are lowest during wet years.
- Orthophosphate is reduced by phytoplankton uptake, which reduces water clarity.

Multiple Regression Analysis

Results from the multiple regression analysis of variables in Table 4 are plotted in a series of bar charts in Appendix C. Figure 5 presents the multiple regression bar charts for total cyanobacteria (PGCS) at the six locations, as it represents the main variable of interest and displays the most fascinating results. Here, green bars to the right represent a positive correlation and red bars to the left represent a negative correlation. If the fit from the linear regression was considered significant ($\alpha = 0.10$) then the bars are shaded darker. The magnitude of each bar represents the beta coefficient (β) or the normalized strength of the correlation.

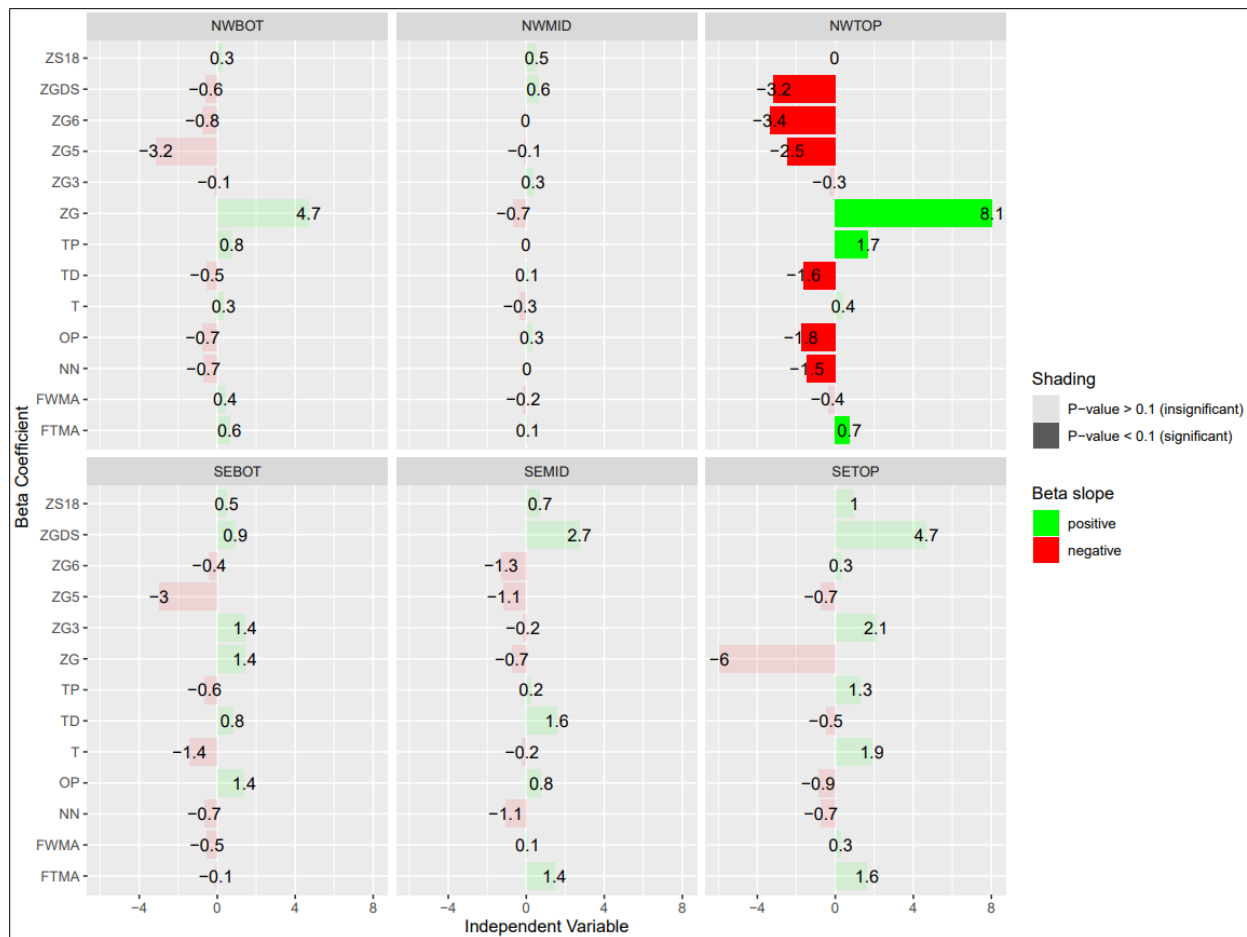


Figure 5. Liberty Lake Multiple Regression Bar Charts for Total Cyanobacteria (PGCS) as the Dependent Variable.

Statistically significant correlations are summarized in Table 5. The only significant correlations observed were by using phytoplankton as the dependent variable. No significant correlations were identified using zooplankton as the dependent variable. No significant correlations were identified at any depth for the SE station (see Appendix C), which is the shallow station and least representative of the main body of the lake.

The clearest result from the multiple regression analysis is the positive correlations of total phosphorus (TP) with total phytoplankton (PG), total cyanobacteria (PGCS), total Bacillariophyta (PS1), and Microcystis (PS9). The negative correlation found between orthophosphate (OP) and PG, PGCS, and PS9 was also similarly found in the PCA analysis, further solidifying this relationship. Both correlations involving phosphorus align with well-known process that as phytoplankton biovolume increases, TP increases as phosphorus is incorporated in the cells and OP decreases as phytoplankton consume it. TP increases do not cause phytoplankton increases.

One key finding was the negative correlation between Daphnia (ZGDS) and cyanobacteria (PGCS) at NWTOP, which implies that either Daphnia are consuming cyanobacteria or the presence of cyanobacteria impairs zooplankton growth. At NWTOP, a positive correlation

between stocked trout mass (FTMA) and PGCS was also found. The previously stated correlations, in addition to the findings from the PCA analysis, support the trophic cascade hypothesis. However, the relative magnitude of the normalized beta slope for FTMA should be considered. Figure 5 shows that trout do have a statistically significant influence but do not have as large of an impact relative to the other significant parameters.

Table 5. Liberty Lake Station-Depth Locations Exhibiting Significant Correlation Between Variables in Multiple Regression Analyses.									
Independent Variable	Dependent Variable								
	PG	PGCS	PS1	PS3	PS9	ZG	ZGDS	ZG3	ZS18
FTMA	-	NWTOP	-	-	-	-	-	-	-
FWMA	-	-	NWBOT	NWMID	-	-	-	-	-
TP	NWBOT	NWTOP	NWBOT	-	NWTOP	-	-	-	-
OP	NWBOT	NWTOP	-	-	NWTOP	-	-	-	-
NN	-	NWTOP	NWBOT	-	-	-	-	-	-
T	-	-	-	-	-	-	-	-	-
TD	-	NWTOP	-	-	-	-	-	-	-
ZG	-	NWTOP	-	-	-				
ZGDS	-	NWTOP	-	-	-				
ZG3	-	-	-	-	-				
ZG5	-	NWTOP	NWBOT	-	-				
ZG6	-	NWTOP	NWBOT	-	-				
ZS18	-	-	NWBOT	-	-				
ZS22						-	-	-	-
PG						-	-	-	-
PGCS						-	-	-	-
PG1						-	-	-	-
PG2						-	-	-	-
PG3						-	-	-	-
PS1						-	-	-	-
PS3						-	-	-	-
PS9						-	-	-	-

Stations shaded green are positive correlation and red are negative correlation. Dashes represent no significant correlations. See Table 2 for variable code definitions.

There were a few counterintuitive results that were identified through this analysis. First, temperature difference (TD) was found to be negatively correlated with cyanobacteria at NWTOP, indicating that weaker stratification increases cyanobacteria in the surface waters. Typically, stratification is thought to support cyanobacteria growth due to their gas vesicles and lower sinking rates than other algae. However, this observation may be explained by a higher phosphorus supply to surface waters from bottom waters during years with weaker stratification since internal loading is a significant source of phosphorus to cyanobacteria in this lake (Tetra Tech 2018).

Second, there is a strong positive correlation ($\beta = +8$) between cyanobacteria biovolume (PGCS) and the abundance of total zooplankton (ZG), which contradicts the moderately strong negative correlation ($\beta = -3$) between cyanobacteria (PGCS) and total Daphnia (ZGDS), copepod nauplii (ZG3), and rotifers (ZG6) at NWTOP (see Figure 5). Generally, these three zooplankton groups represent decreasing size and ability to feed on colonial cyanobacteria and increasing abundance of total zooplankton in lakes. The opposite relationship between zooplankton group and total abundance is counterintuitive but may be an example of why phytoplankton biovolume is better compared to zooplankton biomass than zooplankton number.

Cyanobacteria (PGCS) did not significantly correlate with any variable at the mid or bottom depths at the deep NW station, or at any depth at the shallow SE station (see Figure 5 and Table 5). The observation of significant correlations being limited to only the NWTOP samples may be due to more consistent cyanobacteria abundance in the surface layer of the main body of the lake. For example, cyanobacteria abundance may be more variable at mid and bottom depths due to varied sample depths and vertical migration patterns, and cyanobacteria abundance may be more variable in shallow areas of the lake due to varied wind conditions.

Key findings of the Liberty Lake multiple regression analysis include;

- Significant correlations among parameters was only observed at the main lake NW station and not at the shallow lake NE station, and most of those correlations were in the near surface samples (NWTOP).
- No significant correlations were observed using zooplankton parameters as the dependent variable, indicating that zooplankton populations are not principally controlled by trout stocking or the other parameters included in the multiple regression.
- At NWTOP only, total cyanobacteria biovolume was significantly positively correlated with total zooplankton but negatively correlated with most groups including total Daphnia, copepod nauplii, and rotifers, and this discrepancy may be an artifact of using zooplankton abundance rather than zooplankton biomass in the analysis.
- Total cyanobacteria was also positively correlated with trout stock mass at NWTOP, but no zooplankton or other phytoplankton parameters correlated with trout stock mass. Although these results indicate that trout stocking may stimulate cyanobacteria growth, there was not a clear indication that this was induced by a trophic cascade because of the lack of significant correlations between trout stock mass and zooplankton.
- Cyanobacteria and other phytoplankton groups were significantly positively correlated with total phosphorus and negatively correlated with orthophosphate at the NW station, reflecting the importance of phosphorus uptake by all types of algae in Liberty Lake.

WASHINGTON LAKES DATA ANALYSIS

METHODS

Database Development

A data analysis plan is presented in Appendix B describing what data were available and the initial plan for analyzing the Washington lakes data. A preliminary list of 98 lakes was prepared that included lakes with at least 10 years of available water quality data. However, data could not be obtained for any lakes in eastern Washington (other than Liberty Lake) and for some of the lakes in the four counties in western Washington. Ultimately, complete data sets were obtained for a total of 88 lakes in western Washington that included 30 lakes in Snohomish County, 43 lakes in King County, 6 lakes in Pierce County, and 9 lakes in Thurston County (Figure 6).

The water quality parameters and sampling depths monitored by each county were similar but varied somewhat between each county monitoring program. Each data point had an associated lake, date, and depth. Only values within the study period and that were collected near either the surface (S) or bottom (B) were used for the study. This provided a consistent list of water quality parameters and depths for all 88 lakes. A precipitation parameter was calculated by totaling rainfall from May to October for rain gauges located near each lake. Plankton data were not evaluated because they were not consistently available for most of the western Washington lakes.

Cyanotoxin data were compiled in a spreadsheet from the Washington State Toxic Algae website. Only microcystin and anatoxin-a data were compiled because data for other cyanotoxins were very limited. Data from routine beach samples and the REHAB project (Jacoby et al 2015) were purged from the cyanotoxin database in order to use only data for algae scum samples collected near shore. Undetected results were assigned a value of one-half the method detection limit.

Fish stocking data from 1995 through 2018 were provided in a spreadsheet from WDFW for each of the 88 lakes (J. Spinelli, pers. comm.). Fish species, number, mass, and size were entered by lake and date into a fish database. Areal trout stocking mass was calculated by dividing the total trout mass by the lake surface area (pounds/acre) for use in the data analysis. Private stocking permit application data were also provided for three lakes in King County, but not included in the database because actual stocking amounts are not known. Lake Marcel is the only known lake that applied for a private stocking permit in more than two years and was not also stocked by WDFW.

Extensive data management and processing was done to synthesize all data sets. Mean and maximum values were calculated for four periods: spring (April-June), summer (July-August), fall (September-October) and the year (April-October) for total rain, 11 water quality metrics, and four cyanotoxin metrics. Fish stocking biomass was totaled for each season for trout fry, legal-

size, and all trout for a total of six fish metrics, and longer periods were used for the spring (January-June) and year (January-December) fish stocking mass. The variables used in the analyses of the current study are provided in Table 6.

Table 6. Western Washington Lake Variables.		
Variable Metric	Group	Code
Rain Summer Total	Rain	RAIN-YEAR
Chlorophyll a Surface Mean	WQ	CHLA-SMEAN
Chlorophyll a Surface Max	WQ	CHLA-SMAX
Pheophytin a Surface Mean	WQ	PHEA-MEAN
Secchi Depth Mean	WQ	SECCHI-MEAN
Secchi Depth Min	WQ	SECCHI-MIN
Temperature Surface Mean	WQ	TEMP-SMEAN
Temperature Surface Max	WQ	TEMP-SMAX
Temperature Diff Mean	WQ	TEMPDIFF
Total Nitrogen Surface Mean	WQ	TN-SMEAN
Total Phosphorus Surface Mean	WQ	TP-SMEAN
Total Phosphorus Bottom Mean	WQ	TP-BMEAN
Microcystin Mean	Toxin	MC-MEAN
Microcystin Max	Toxin	MC-MAX
Anatoxin a Mean	Toxin	AT-MEAN
Anatoxin a Max	Toxin	AT-MAX
Trout Total Mass-Annual	Trout	TTM-YEAR
Trout Total Mass-Spring	Trout	TTM-SPRING
Trout Total Mass-Fall	Trout	TTM-FALL
Trout Areal Mass-Annual	Trout	TAM-YEAR
Trout Areal Mass-Spring	Trout	TAM-SPRING
Trout Areal Mass-Fall	Trout	TAM-FALL

Data Analysis

Analysis of western Washington lakes data included reviewing chronological plots and conducting multiple regression analysis of key parameters. Principle component analysis was not conducted on the Washington lakes data set because of the large number of lakes and limited number of parameters. Correlation analysis was not conducted prior to the multiple regression analysis because the selected key parameters were assumed to be independent based on results of the Liberty Lake correlation analysis.

Temporal and Spatial Patterns

Chronological plots were made for each sampling date including three key parameters: areal trout stocking mass, chlorophyll-a concentration, and microcystin concentration. These plots were reviewed to identify possible data entry errors and trends amongst parameters by visual inspection.

Multiple Regression Analysis

A multiple regression analysis was conducted for every lake with a complete record of key parameters in the database. Initially, multiple regression was performed with all 22 variables in the database (see Table 6). However, many variables were eliminated from subsequent analyses because of incomplete records resulting in less than six years of data for more than 50 percent of the 88 lakes. In addition, anatoxin-a was not included in the subsequent analyses due to a lack of detected values among the lakes. Ultimately, multiple regression was simplified and performed for four dependent variables with five independent variables. The dependent variables of interest included:

- Mean and maximum surface chlorophyll concentrations (CHLA-SMEAN and CHLA-SMAX)
- Mean and maximum scum microcystin concentrations (MC-MEAN and MC-MAX).

The independent variables included:

- Total rainfall (RAIN-YEAR).
- Mean surface water temperature (TEMP-SMEAN)
- Mean Secchi depth (SECCHI-MEAN)
- Mean surface total phosphorus concentration (TP-SMEAN)
- Total trout stocking mass (TAM-YEAR)

Multiple regression was performed separately for spring data (April-June) and annual data (April-October) for all variables except trout stocking data were based on the entire calendar year. Spring data were analyzed separately to see if more significant correlations with fish stocking occur in the spring when the majority of trout are stocked in western Washington lakes compared to annual values.

RESULTS

Temporal and Spatial Patterns

Seasonal mean concentrations of chlorophyll-a and microcystin are plotted as points (o for chlorophyll-a and x for microcystin), and annual areal trout mass are plotted as purple bars for each year at each lake in Appendix D. Figure 7 presents the chronological plots for Lake St. Clair and Steel Lake, which show examples of two lakes that experience spring (green symbols) and fall (brown symbols) algae blooms, respectively. These plots are useful for identifying data gaps. For example, chlorophyll-a data are missing for Lake St. Clair from 2019 to 2019 and for Steel Lake from 2005 to 2013. Microcystin testing did not begin for most lakes in Washington until 2008.

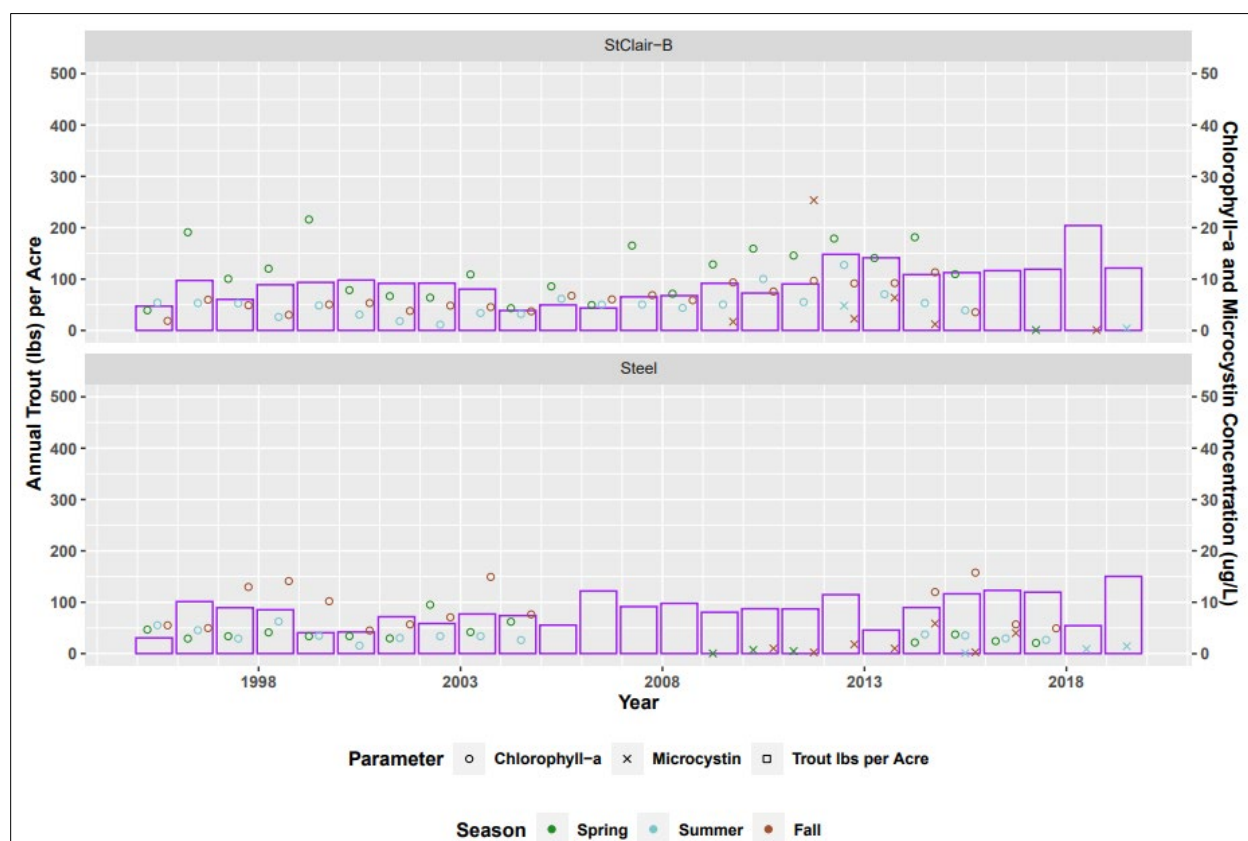


Figure 7. Seasonal Means for Chlorophyll-a and Microcystin as Points and Annual Total Trout Stocking Mass as Bars for Lake Saint Clair and Steel Lake.

As expected, chronological patterns vary widely among the lakes, and often vary widely within one lake for chlorophyll-a and microcystin but much less so for trout stocking. Annual trout stocking amounts were typically less variable than mean chlorophyll-a and microcystin concentrations. These plots are thought to be particularly useful for lake managers to depict

seasonal and annual patterns in chlorophyll-a and microcystin concentrations in comparison to annual trout stocking amounts.

Multiple Regression Analysis

Results of the multiple regression analysis of the western Washington lakes data are presented as bar charts for each lake in Appendix D that include charts for each dependent variable using annual data followed by charts for each dependent variable using spring data. Figure 8 shows an example of the results from six of the 88 lakes presented in Appendix D using the spring maximum surface chlorophyll-a concentration (CHLA-SMAX) as the dependent variable.



Figure 8. Multiple Regression Analysis Results for Spring Maximum Chlorophyll in Six Washington Lakes.

Parameter correlations found significant for spring were not always significant in the annual data and vice versa. For example, Cottage Lake shows mean chlorophyll and trout stocking were positively correlated for annual data but not spring data. This example shows that although most trout are stocked in lakes soon before the opening day of fishing season in late April, it can be more correlated with annual peaks in algae than spring peaks in algae.

Annual Data

Multiple regression analysis results using annual data (April-October) are summarized in Table 7 for chlorophyll-a and in Table 8 for microcystin. Here, the results are grouped into three types of lakes: all trout planted lakes, high trout stocking lakes, and no trout planted lakes. High trout planted lakes include only those trout planted lakes where the average areal trout stocking mass exceeds the median value for all trout planted lakes. Comparison of non-trout variable results among these three types of lakes potentially shows an overall effect of trout stocking on these variables.

Table 7. Percent of Lakes with Significant Variable Correlations with Annual Mean and Maximum Chlorophyll-a Concentrations for Three Types of Trout Stocking.												
Independent Variable	All Trout Stocked (n=61)				High Trout Stocked (n=35)				No Trout Stocked (n=18)			
	CHLA-SMEAN		CHLA-SMAX		CHLA-SMEAN		CHLA-SMAX		CHLA-SMEAN		CHLA-SMAX	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG
Trout	7%	3%	3%	8%	9%	3%	6%	3%	-	-	-	-
TP Surf	15%	0%	16%	0%	11%	0%	14%	0%	17%	0%	11%	0%
Secchi	2%	28%	2%	21%	0%	26%	0%	20%	0%	17%	6%	11%
Temp Surf	2%	8%	5%	12%	3%	3%	3%	12%	0%	6%	6%	0%
Rain	5%	3%	5%	3%	0%	3%	3%	0%	0%	0%	6%	6%

Table 8. Percent of Lakes with Significant Variable Correlations with Annual Mean and Maximum Microcystin Concentrations for Three Types of Trout Stocking.												
Independent Variable	All Trout Stocked (n=24)				High Trout Stocked (n=11)				No Trout Stocked (n=4)			
	MC-MEAN		MC-MAX		MC-MEAN		MC-MAX		MC-MEAN		MC-MAX	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG
Trout	4%	4%	0%	4%	9%	0%	0%	0%	-	-	-	-
TP Surf	4%	0%	4%	0%	9%	0%	9%	0%	0%	0%	0%	0%
Secchi	8%	4%	8%	4%	9%	0%	9%	0%	0%	0%	0%	0%
Temp Surf	4%	0%	4%	0%	9%	0%	9%	0%	0%	0%	0%	0%
Rain	4%	4%	4%	4%	0%	9%	0%	9%	25%	0%	25%	0%

Chlorophyll-a concentrations were most correlated with total phosphorus among all three types of lakes, as would be expected. Mean chlorophyll-a was significantly positively correlated with surface total phosphorus in 15 percent of the 61 trout stocked lakes, 11 percent of the 35 high trout stocked lakes, and 17 percent of the 18 unstocked lakes. Maximum chlorophyll-a was significantly positively correlated with surface total phosphorus in 16 percent of the 61 trout stocked lakes, 14 percent of the high trout stocked lakes, and 11 percent of the 18 unstocked lakes. Thus, significantly positive chlorophyll-a/total phosphorus correlations were less frequent in high stocked lakes than unstocked lakes for mean chlorophyll-a but not for maximum chlorophyll-a.

Secchi depth also exhibited consistent significant relationships among all three types of lakes. Mean chlorophyll-a was significantly negatively correlated with Secchi depth in 28 percent of the trout stocked lakes, 26 percent of high trout stocked lakes, and 17 percent of the unstocked lakes. Maximum chlorophyll-a was significantly negatively correlated with Secchi depth in 21 percent of the trout stocked lakes, 20 percent of high trout stocked lakes, and 11 percent of the unstocked lakes. Thus, significantly negative chlorophyll-a/Secchi depth correlations were more frequent in high stocked lakes for both mean and maximum chlorophyll-a. Chlorophyll-a was positively correlated with Secchi depth for a few lakes but was not negatively correlated with total phosphorus in any lakes.

Surface temperature and annual rain correlated either positively or negatively with chlorophyll-a among relatively few lakes. No clear relationship between surface temperature or rain with chlorophyll-a was identified through this analysis.

The correlations between stocked trout and chlorophyll were relatively infrequent and partially contradict each other. A higher percentage of all trout stocked lakes showed positive than negative correlations between trout and mean chlorophyll-a (7 percent positive versus 3 percent negative), and high trout stocked lakes showed even more frequent positive correlations (9 percent positive versus 3 percent negative). The relationship between trout and maximum chlorophyll-a varied depending on whether it was trout stocked or high trout stocked. These inconsistencies could be due to variability between lakes or simply that there is no direct relationship to trout stocking and that these correlations are the result of other coincident ecological processes.

Microcystin was correlated with independent variables only for lakes where microcystin concentrations have exceeded the historical state guideline of 6 µg/L (which was recently increased to 8 µg/L). Thus, only 24 stocked lakes, 11 high stocked lakes, and 4 unstocked lakes were tested for microcystin (see Table 8) compared to 61 stocked lakes, 35 high stocked lakes, and 18 unstocked lakes for chlorophyll-a (see Table 7). When microcystin was analyzed as the dependent variable there were less significant correlations with the independent variables compared to chlorophyll, and they were often inconsistent and contradicting. Mean and maximum microcystin were positively correlated with total phosphorus in only 4 percent of the stocked lakes, 9 percent of the high stocked lakes, and none of the unstocked lakes. Microcystin was more often positively correlated than negatively correlated with Secchi depth among stocked lakes (8 percent positive versus 4 percent negative) and high trout stocked lakes (9 percent positive versus 0 percent negative), and in none of the unstocked lakes.

Mean microcystin was equally positively correlated (4 percent) and negatively correlated (4 percent) with trout mass, while maximum microcystin was only negatively correlated (4 percent) with trout mass in all trout stocked lakes. Among high trout stocked lakes, 9 percent of the lakes were positively correlated with mean microcystin and 0 percent were positively correlated with maximum microcystin. Thus, microcystin was correlated with total phosphorus, Secchi depth, and trout mass in only 1 of the 11 high stocked lakes and none of the 4 unstocked lakes using annual data.

Spring Data

Multiple regression analysis results using spring data are summarized in Table 9 for chlorophyll-a and in Table 10 for microcystin that are also grouped into all trout planted lakes, high trout stocked lakes, and no trout planted lakes. Less than 50 percent of the lakes analyzed using annual data had enough spring data for analysis, reducing the significance of the spring data analysis findings.

Table 9. Percent of Lakes with Significant Variable Correlations with Spring Mean and Maximum Chlorophyll-a Concentrations for Three Types of Trout Stocking.

Variable	All Trout Planted (n=22)				High Trout Planted (n=13)				No Trout Planted (n=14)			
	CHLA-SMEAN		CHLA-SMAX		CHLA-SMEAN		CHLA-SMAX		CHLA-SMEAN		CHLA-SMAX	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG
Trout	9%	5%	18%	0%	15%	8%	31%	0%	-	-	-	-
TP Surf	14%	0%	23%	0%	8%	0%	15%	0%	7%	0%	7%	0%
Secchi	0%	55%	0%	36%	0%	69%	0%	38%	7%	21%	0%	14%
Temp Surf	0%	5%	0%	9%	0%	8%	0%	15%	14%	0%	21%	7%
Rain	0%	5%	5%	9%	0%	8%	8%	15%	0%	0%	0%	0%

Table 10. Percent of Lakes with Significant Variable Correlations with Spring Mean and Maximum Microcystin Concentrations for Three Types of Trout Stocking.

Variable	All Trout Planted (n=8-11)				High Trout Planted (n=4-6)				No Trout Planted (n=2)			
	MC-MEAN		MC-MAX		MC-MEAN		MC-MAX		MC-MEAN		MC-MAX	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG	POS	NEG
Trout	9%	9%	9%	9%	0%	17%	0%	17%	-	-	-	-
TP Surf	14%	0%	14%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Secchi	38%	13%	38%	13%	40%	20%	40%	20%	0%	0%	0%	0%
Temp Surf	0%	13%	0%	13%	0%	20%	0%	20%	0%	0%	0%	0%
Rain	0%	9%	0%	9%	0%	0%	0%	0%	0%	0%	0%	0%

As found in the annual data, the spring data also exhibited standard relationships between chlorophyll-a to TP and Secchi depth. Although there are some contradictions, overall, these relationships hold. Interestingly, there were a substantial proportion of unstocked lakes showing positive correlations of mean and maximum spring chlorophyll-a with surface water temperature (14 and 21 percent, respectively) that was not observed in any lakes stocked with trout. This season-specific pattern suggests that algae biomass may be affected by how much a lake warms in the spring since growth rates generally increase with increased water temperature, but temperature effects are minimized if the lake is stocked with trout.

Contrary to the annual mean values, the spring data set displayed results that partially support the trophic cascade hypothesis of trout stocking increasing spring algal blooms. Most trout stocking occurs in the spring (April – June) and would be expected to have a greater immediate impact than over the entire annual period (April – October). Maximum spring chlorophyll-a significantly positively correlated with trout mass in 18 percent of all trout stocked lakes and 31 percent of high trout stocked lakes, and did not negatively correlate with trout mass in any of the lakes. Mean spring chlorophyll-a was less correlated with trout mass and with mixed results showing significantly positive and negative relationships. Thus, trout stocking rates may affect the size of spring algae blooms in some lakes.

Spring mean/maximum microcystin correlation analysis results were like those observed for annual data and with typically higher percentages among the fewer lakes tested. Among the 8 to 11 trout planted lakes tested, microcystin was most often positively correlated with total phosphorus and Secchi depth, and negatively correlated with temperature. The positive correlation of spring Secchi depth with microcystin with versus the expected and observed negative correlation with chlorophyll-a suggests that spring toxin production may be greater when less algae are present in some lakes. Contrary to the relationship with chlorophyll and the trophic cascade hypothesis for cyanobacteria, trout stock mass was not positively correlated with microcystin in any of the high trout stocked lakes and in fact was negatively correlated in 1 of 6 high trout stocked lakes.

Algae-Trout Correlated Lakes

Lakes where chlorophyll-a or microcystin concentrations were significantly correlated with trout stocking rates are presented in Table 11. The lakes are grouped by annual trout stocking rates on an areal basis where the median rate for all stocked lakes was used to separate low and high stocked lakes. This presentation shows that positive correlations were typically limited to high stocked lakes and negative correlations were typically limited to low stocked lakes. Exceptions include positive correlations for the low stocked Black Lake, and negative correlations for the high stocked Shady and Ward lakes.

Table 11. Lakes Showing Significant Correlations of Chlorophyll-a and Microcystin with Trout Stocking.

Trout Stocking Class	County	Name	Annual				Spring			
			Chlorophyll-a		Microcystin		Chlorophyll-a		Microcystin	
			Mean	Max	Mean	Max	Mean	Max	Mean	Max
None	Pierce	Louise		-						
Low	King	Dolloff	-	-						
Low	King	Killarney	-	-						
Low	Snohomish	Chain	-							
Low	Snohomish	Loma			-	-				
Low	Snohomish	Martha-S		-						
Low	Thurston	Black-S	+						+	+
High	King	Alice						+		
High	King	Cottage	+	+				+		
High	King	Morton					+	+		
High	King	Shady					-			
High	King	Wilderness	+		+					
High	Thurston	Saint Clair	+	+			+	+		
High	Thurston	Ward							-	-

Cells shaded green are positive correlation and red are negative correlation. Empty cells represent no significant correlation. Trout stocking classification is based on the annual median areal trout mass among all lakes where high stocking lakes are greater than the median, low stocking lakes are less than the median, and none are not stocked.

CONCLUSIONS AND RECOMMENDATIONS

LIBERTY LAKE

Eighteen years of extensive water quality, phytoplankton, zooplankton, and fish stocking data were used to evaluate the impacts of fish stocking on cyanobacteria blooms in Liberty Lake, Washington. To assess the potential causes of cyanobacteria blooms this robust data set was analyzed using statistical methods including principle component analysis, Kendall's Tau correlation analysis, and a multiple regression analysis.

Key findings of the principle component analysis include:

- Liberty Lake exhibits standard trophic interactions with increased trout stocking decreasing zooplankton and increasing phytoplankton.
- Stocking of legal-sized trout reduce zooplankton more than stocking of trout fry.
- Total phosphorus increases with trout stocking, possibly due to phosphorus in trout excretion or from increased phytoplankton biomass.

Key findings of the multiple regression analysis include:

- Significant correlations among parameters were only observed at the main lake station NW and most of those correlations were in the near surface samples (NWTOP).
- No significant correlations were observed using zooplankton parameters as the dependent variable, indicating that zooplankton populations are not principally controlled by trout stocking or the other parameters included in the multiple regression.
- At NWTOP only, total cyanobacteria biovolume was significantly positively correlated with total zooplankton but negatively correlated with most groups including total *Daphnia*, copepod nauplii, and rotifers, and this discrepancy may be an artifact of using zooplankton abundance rather than zooplankton biomass in the analysis.
- Total cyanobacteria biovolume was also positively correlated with trout stock mass at NWTOP, but no zooplankton or other phytoplankton parameters correlated with trout stock mass. Although these results indicate that trout stocking may stimulate cyanobacteria growth, there was not a clear indication that this was induced by a trophic cascade because of the lack of significant correlations between trout stock mass and zooplankton abundance.
- Cyanobacteria and other phytoplankton groups were significantly positively correlated with total phosphorus and negatively correlated with orthophosphate at the NW station, reflecting the importance of phosphorus uptake by all types of algae in Liberty Lake.

WESTERN WASHINGTON LAKES

Testing the trophic cascade hypothesis as it relates to trout stocking on cyanobacteria in western Washington lakes as was performed by combining routine monitoring data with cyanotoxin and trout stocking data for 88 lakes in Snohomish, King, Pierce, and Thurston counties. Relationships between key variables were explored using multiple regression analyses of spring (April – June) and annual (April-October) datasets.

The results showed extensive variability among parameters and between lakes. The only clear statistically significant relationships were between chlorophyll, total phosphorus, and water clarity (Secchi depth), as would be expected from limnological principals.

Overall, no strong conclusion could be made regarding stocked trout inducing algae blooms or cyanotoxin production. However, the analysis results indicate that algae blooms or cyanotoxin production may be significantly affected by trout stocking in some lakes. Significantly positive correlations between trout stocking amounts and chlorophyll or microcystin were observed in 6 of the 61 stocked lakes and 5 of those lakes were stocked at higher rates than most of the other lakes. However, significantly negative correlations between trout stocking amounts and chlorophyll or microcystin were observed in 7 of the 61 stocked lakes but only 2 of those lakes were stocked at higher rates than most of the other lakes.

One limiting factor in this analysis is that trout stocking rates did not vary much between years for each lake. Significant correlations are difficult to detect without a wide range of values for each variable.

Although a conclusion could not be made that is generalized for all western Washington lakes, it may be that certain lakes are more susceptible to trophic alterations from stocked trout. Trout stocking may not have a significant top down control on primary production but results of this study support consideration of reducing stocking rates for some lakes that are prone to cyanobacteria blooms.

MANAGEMENT RECOMMENDATIONS AND FUTURE WORKS

No changes in the trout stocking program are recommended at this time based on the findings of this study. Additional data collection and analysis would be needed to provide a scientific basis for changing the trout stocking program in Washington or for a specific lake.

A next step in the data analysis could be to further investigate which lake characteristics are associated with the observed positive correlations between trout stocking and algae blooms. With a smaller sample size of six lakes showing positive correlations and seven lakes showing negative correlations, a more in-depth analysis could be conducted.

Additional analysis of the database compiled for this study could provide more meaningful results. In particular, the western Washington data could be analyzed further using different

metrics associated with the trout size (e.g., legal versus jumbo sizes) and stocking timing (e.g., spring versus fall) with respect to immediate effects on chlorophyll-a, cyanotoxins, and nutrients. Additional independent variables could be included in the analysis such as lake size and depth, stratification stability, residence time, watershed to lake ratio, and percent imperviousness or development of the watershed. Synthesizing results from the present study and additional analyses could be further analyzed using a decision tree to predict the effects of trout stocking relative to other factors on algae blooms and cyanobacteria toxin production. Factors controlling algae blooms and toxin production are complex and are expected to vary greatly between lakes with different physical, chemical, and biological characteristics. Resident fish and aquatic macrophyte populations are examples of factors that likely impact algae blooms but are often not well characterized.

Lakes are highly dynamic ecosystems that can change significantly at daily time scales. The data sets used here do show many trends at large time scales, but sparse and averaged data sets do not accurately represent these dynamic systems. To fully understand the impacts of trout stocking, a more robust field study would be required. Trout are normally stocked in spring, which is typically the most dynamic period for plankton in lakes due to increasing temperatures and light for growth. Ideally data for the key parameters identified in this study would be collected at higher frequencies before and after trout stocking, would include additional key parameters such as zooplankton species biomass, and would include more variation in trout stocking rates within each lake studied than typically occurs.

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APPENDIX A

Literature Review

TECHNICAL MEMORANDUM

Date: November 28, 2018

To: BiJay Adams and Jeremy Jenkins, Liberty Lake Sewer and Water District

Copy to: Lizbeth Seebacher, Washington Department of Ecology; Randall Osborne and Justin Spinelli, Washington Department of Fish and Wildlife; Joan Hardy, Washington Department of Health (retired); Daniel Schindler and Mike Brett, University of Washington; Tom Brattebo, Liberty Lake Resident; Chris Knutson, King County Water and Land Resources Division; Isabel Ragland, Pierce Conservation District; Marisa Burghdoff, Snohomish County Surface Water Management; and Jane Mountjoy-Venning, Thurston County Public Health and Social Services

From: Ellen Preece, Robertson-Bryan, Inc. and Rob Zisette, Herrera Environmental Consultants

Subject: Literature Review of Trout Stocking Effects on Harmful Algae Blooms

INTRODUCTION

This memorandum presents a summary of a literature review conducted for the Trophic Cascade Effects on Algae Blooms in Washington Lakes Project. This memorandum is distributed to the project technical committee for Task 2 of the project in accordance with the Water Quality Algae Control Program Agreement between the Washington Department of Ecology and the Liberty Lake Sewer and Water District (Agreement No. WQALG-2019-LibWSD-00018).

The goal of this literature review is to summarize findings of relevant studies on the trophic cascade effects of fish management on phytoplankton in lakes to guide the data analysis and for comparison of the study results to findings of other relevant studies. The focus of the literature review is on the effects of trout stocking on harmful algae blooms. The literature review findings are summarized separately for the following topics:

- Survival, Growth, and Creel of Stocked Trout
- Trout Diet and Size Class
- Trout Stocking and Lake Food Webs
- Trout Stocking and Nutrients



The summary is followed by a bibliography and abstracts for the reviewed literature. A separate bibliography is presented for other literature on the general topic of trophic cascades. Trophic cascades are powerful indirect interactions that can control entire ecosystems, occurring when predators in a food web suppress the abundance or alter the behavior of their prey, thereby releasing the next lower trophic level from predation (or herbivory if the intermediate trophic level is a herbivore) (Wikipedia). Trophic cascades are indirect species interactions that originate with predators and spread downward through food webs. The term “trophic cascade” applies both to the dynamic response of the distribution and abundance of species to a change in the driving predator and to the dynamic processes that maintain the distribution and abundance of species in static or stationary systems (Ripple et al. 2016). An example diagram of a trophic cascade in a lake is presented in Figure 1 (Walsh et al. 2016).

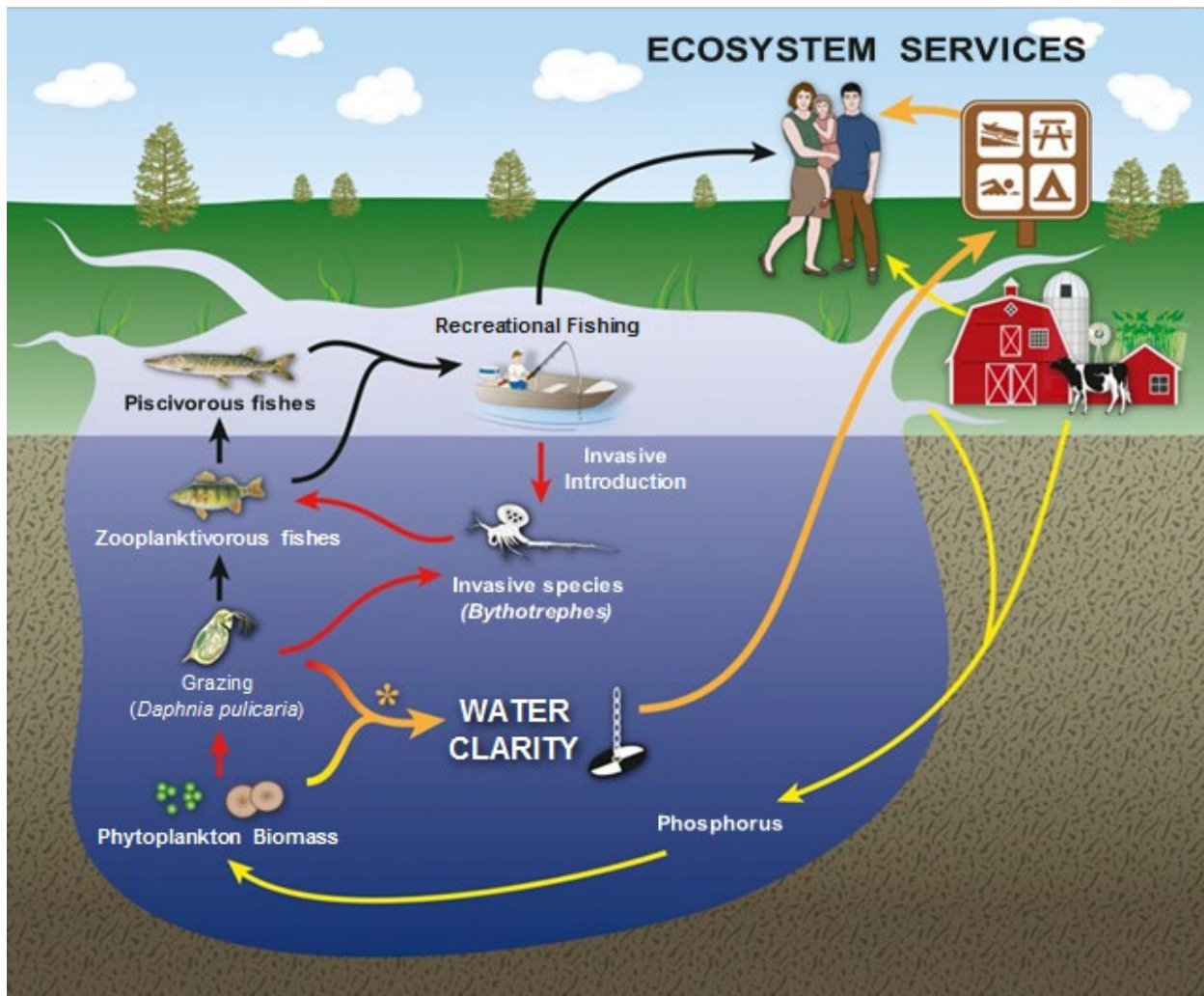


Figure 1. Lake Trophic Cascade Diagram Example (Walsh et al. 2016).

TROUT STOCKING LITERATURE REVIEW SUMMARY

Survival, Growth, and Creel of Stocked Trout

A number of factors affect the survival, growth, and catch rates (creel) of stocked trout. Factors include: size class stocked (Gunn 1987), competition with resident fish (Gunn 1987), intraspecific feeding competition (Skinner et al. 2014), environmental conditions (Koenig and Meyer 2011), strain (Ayles and Baker 1983), stocking density (Miko et al. 1995), stocking season (Yule et al. 2000), and angler pressure. Because the factors that affect survival and growth are multifaceted, it is difficult to determine the factors that influence fish returning to creel in inland waters (Losee and Philips 2017). In western Washington lakes, a 1992 Fish and Wildlife Report found less than 20 percent of stocked rainbow trout fry returned to creel compared with 70 percent in eastern Washington Lakes (Jackson and Lovegren 1992 as cited in Losee and Phillips 2017). The source of variation between eastern and western Washington creel data was not discussed. However, it is recognized that environmental conditions (e.g., temperature, acidity, dissolved oxygen) of western Washington lakes are not ideal for supporting the long-term survival of rainbow trout (Losee and Philips 2017). This is one possible explanation for the lower creel in western Washington compared to eastern Washington.

It is well understood that anglers prefer to catch larger fish. Losee and Philips (2017) found larger fish (>280 mm) recruit to a fishery at a greater rate than smaller fish (<280 mm) in western Washington Lakes, in part because anglers preferred to catch the larger fish. Although it is possible for smaller fish to eventually benefit sport fisheries once they reach a larger size, due to the suboptimal environmental conditions in western Washington lakes smaller fish are unlikely to support a put-and-take fishery (Losee and Philips 2017).

Trout Diet and Size Class

Farmed fish are typically raised on a high-energy diet consisting of fish meal, fish oil, and various fillers, vitamins and minerals depending on the brand of food (Martin et al. 2018). After fish are stocked into lakes their diet immediately switches to food sources within the lake. Trout are opportunistic feeders that rely on wide variety of prey from zooplankton and small insects, to crayfish and smaller fish. Microcrustaceans, particularly cladocerans and chironomids, are typically important components of trout diets (Hanisch 2016). A study in Twin Lakes, Washington, compared the diet of different size classes of brook trout and rainbow trout (i.e., <100 mm, 100 to 199 mm, 200 to 299 mm, and >299 mm) (Christensen and Moore 2007; Skinner 2014). Skinner et al. (2014) found size class did not affect prey choices and that brook trout and rainbow trout prey primarily consisted of Daphnia, dipterans, and amphipods with occasional predation on Golden Shiners by rainbow trout (Skinner et al 2014). Similarly, a study in Canadian boreal lakes found the frequency of planktivory was independent of trout size (Hanisch et al. 2016). In contrast, a study in New Zealand lakes found subadult and small adult (200 to 400 mm) rainbow trout consumed a less diverse variety of prey items than large adult rainbow trout (>400 mm). Yet, due to a small sample size diets between size classes were not

statistically significant (Blair et al. 2012). In the Italian Alps, introduced adult brook trout were found to rarely feed on zooplankton while zooplankton comprised the majority of juvenile brook trout's diet (Tiberti et al. 2014a). Nonetheless, it was adults that had the greatest impact on zooplankton communities, because when large bodied zooplankton were available the prey became an important temporary food resource for the adults.

The studies reviewed above suggest that in general adult and juvenile trout rely on similar prey sources for their diets, but adult trout may have a larger impact on zooplankton populations than juvenile trout. Due to the limited number of studies that address how size class affects prey choices it is not possible to make any definitive conclusions on how different sized stocked fish may affect prey resources within lakes.

Trout Stocking and Lake Food Webs

Most research on the effects of trout stocking on lake food-webs has occurred in fishless headwater lakes and streams (Dunham et al. 2004; Eby et al. 2006). These systems are typically characterized by low species richness and low habitat complexity and have a low resistance to fish introductions. As such, stocked trout in these systems negatively affect native biota (Knapp et al. 2005; Gozlan 2008). Although fewer studies have addressed impacts of stocking on the food web of more productive lakes, those that have addressed less pristine systems have found that long-term fish stocking (i.e., up to 50 years) has minimal to no effect on benthic invertebrate assemblages. For example, researchers in New Zealand found no differences in overall benthic biomass in lakes with introduced trout compared with troutless lakes (Wissinger et al. 2006). Similar effects were also noted in Canadian boreal foothill lakes where trout were stocked for recreational angling. There were no clear adverse effects of the stocked trout on invertebrate community composition, although a few taxa had either increased or decreased abundance due to fish stocking (Nasmith et al. 2013). In another Canadian study, boreal lakes were studied 1 year before and 2 years after trout stocking. Here, biomass but not abundance of littoral invertebrate assemblages were affected by the stocking (Hanisch et al. 2013). However, one taxon, Chironomidae, were consumed more frequently after stocking. This led to significantly smaller (i.e., shorter) sized Chironomidae compared to pre-stocking conditions. Fish stocking was also found to affect Chironomidae in another Canadian Boreal lake study. Some density and minor compositional differences in *Chaoborus* were noted between stocked and unstocked lakes suggesting that trout may have limited effects on *Chaoborus* (Holmes et al. 2017; Lancelotti et al. 2016).

Trout stocking can also strongly influence microcrustaceans communities in unproductive and naturally fishless mountain lakes. A number of studies in these relatively pristine lakes have found stocked trout lead to reductions in abundance and mean body size of microcrustacean taxa (e.g., Parker et al. 2001; Tiberti et al. 2014a). Studies have also found when trout are present in a lake, regardless of if they are stocked or native, that smaller cladocerans and calanoids dominate the microcrustacean population relative to fishless lakes (Holmes et al. 2017; Lancelotti et al. 2016).

Studies addressing trout stocking in productive lakes, or lakes with a history of fish stocking, and the subsequent effects on microcrustacean communities are rare in the literature. Available studies report various effects of fish stocking on zooplankton communities suggesting that stocking impacts are not universal across inland waterbodies. In a Canadian boreal lake study ($n = 14$ lakes), effects of non-native trout on microcrustacean zooplankton were compared in stocked, unstocked (but fish-bearing), and fishless lakes. Except for taxon richness which was greater in stocked lakes, zooplankton metrics generally showed limited to no differences between stocked and unstocked lakes (Holmes et al. 2017). In contrast, fishless lakes were dominated by larger cladoceran and calanoid taxa relative to fish-bearing lakes (Holmes et al. 2017).

Stocking had a much greater effect on the zooplankton community in Square Lake, Minnesota. Here, rainbow trout predation on large-bodied *Daphnia* (*Daphnia pulicaria*) was identified as the most likely cause for the declining water transparency (Hembre 2016). This was confirmed after a 3-year stocking moratorium when densities of *D. pulicaria* were significantly greater compared to the pre-moratorium years when rainbow trout were stocked in the lake. Biomass concentrations of the smaller bodied *Daphnia mendotae* were significantly lower in years the lake was not stocked, likely because the larger bodied *Daphnia* are superior competitors to smaller bodied zooplankton. The difference between stocking and non-stocking years was most pronounced in spring (April and May) likely because the *D. pulicaria* population would proliferate in response to the spring phytoplankton bloom (Hembre 2016).

It is possible that zooplankton responses to stocked fish vary between systems due to the unique features of each waterbody. It is also possible that the number of fish stocked into waterbody are responsible for changes to microcrustacean food webs. For example, Blair et al. (2014) reports that stocked sports fish can have substantial effects on prey abundance, especially if fish are overstocked. Similarly, Skinner et al. (2014) suggests overstocking may lead to intraspecific competition for food resources between trout species (Skinner et al. 2014). This intraspecific competition may lead to greater impacts on certain zooplankton species and thus affect the entire food web of a lake.

Trout Stocking and Nutrients

The impacts of fish stocking on nutrients and algal productions has also received little attention. In the relatively pristine lakes of the Sierra Nevada, California, a study found that introduced trout fundamentally altered nutrient cycles and stimulated primary production (Schindler et al. 2001). Stocked trout were found to access and distribute benthic phosphorus (P) sources that were not formally available to pelagic communities of oligotrophic mountain lakes (Schindler et al. 2001). The researchers also found that trout grew faster and excreted significantly more P in currently stocked lakes compared to lakes that contained introduced, but self-sustaining trout populations.

In Square Lake, MINNESOTA, stocked trout were also found to influence nutrient cycling. A stocking moratorium in Square Lake resulted in lower surface water algae biomass, significantly

clearer water, and significantly lower TP in surface waters compared to pre-moratorium years (Hembre 2016). The authors hypothesize that lower TP was a result of the increased standing stock of the large bodied *Daphnia* due to a reduction in predation pressures. In contrast, a study on Canadian boreal lakes (stocked lakes, unstocked lakes, and fishless lakes) found Chl-a, Chl-a:TP, nor Chl-a:TN differed among lake types (Holmes et al. 2017).

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Abstracts

Alexiades et al. 2017

Each year, millions of hatchery-raised fish are stocked into streams and rivers worldwide, yet the effects of hatchery-raised fish on stream nutrient cycles have seldom been examined. We quantified the influence of supplemental non-native fish stocking, a widespread recreational fishery management practice, on in-stream nutrient storage and cycling. We predicted that supplemental, hatchery-raised brown trout (*Salmo trutta*) stocking would result in increased N and P supply relative to in-stream biotic demand for those nutrients and that stocked fishes would remineralize and store a significantly greater amount of N and P than the native fish community, due to higher areal biomass. To test these predictions, we measured the biomass, nutrient ($\text{NH}_4^+\text{-N}$ and soluble reactive phosphorus [SRP]) remineralization rates, and body

carbon, nitrogen, and phosphorus content of the native fish community and trout stocked into four study streams. We then estimated fish growth rates to determine species-specific nutrient sequestration rates in body tissues for both stocked and native fish and measured ammonium and phosphorus uptake rates to determine the relative influence of net fish nutrient remineralization on stream nutrient cycles. When brown trout were stocked in these systems at density levels that were orders of magnitude higher than ambient native fish density, they provided a sizeable source of NH_4^+ -N that could account for up to 85 percent of demand for that nutrient. Stocked trout had minimal effects on in-stream SRP cycles even at high release densities, likely due to low per capita SRP excretion rates. A unique feature of our study was that we evaluated the temporal component of the stocked trout nutrient subsidy by estimating the number of fish removed from the system through natural mortality and angler harvest, which indicated that the stocked trout subsidy lasted approximately 6 to 8 weeks after stocking. By combining population models with areal nutrient excretion rates and estimates of biotic nutrient uptake, we showed that trout stocking provided a strong pulsed nutrient subsidy.

Ayles et al. 1983

Several thousand small, shallow, highly productive lakes across the Canadian prairies are used by farmers for commercial and recreational production of rainbow trout. This report summarizes the results of evaluations of genetic differences between strains of trout from 1972 to 1978. Strains of fish were obtained from the wild and from private and government hatcheries in Canada, the USA, and Europe. Matched plantings of different strains were made in several experimental lakes annually and growth and survival were determined. The relative effects of strains (genetic effect), lakes (environment effects) and strain \times lake interactions were determined in each study. Significant hybrid vigor for both growth and survival was observed for some but not all crosses.

Blair et al. 2012

We characterized seasonal and ontogenetic changes in diet and prey energy density of rainbow trout (*Oncorhynchus mykiss*) in Lake Rotoiti, New Zealand, to better understand the prey requirements of trout in central North Island lakes. Common smelt (*Retropinna retropinna*) was the dominant prey item of rainbow trout larger than 200 mm (77.8 percent of diet by weight), followed by kōura (freshwater crayfish [*Paranephrops planifrons*]; 6.3 percent), common bully (*Gobiomorphus cotidianus*; 5.5 percent), and koaro (*Galaxias brevipinnis*; 3.4 percent). Juvenile rainbow trout (B200 mm) consumed amphipods, aquatic and terrestrial insects, oligochaetes, tanaid shrimps, and smelt. Trout consumed koaro only in autumn and winter; consumption of other species did not vary seasonally. The maximum size of smelt consumed increased with increasing trout size, but trout continued to consume small smelt even as large adults. Consumption of larger prey items (koaro and kōura) also increased with increasing trout size. This study indicates the importance of smelt for sustaining rainbow trout populations, as predation on other species was relatively low. These findings provide a basis for bioenergetic modelling of rainbow trout populations in lakes of the central North Island of New Zealand.

Blair et al. 2013a

To predict potential effects of climate and anthropogenic impacts on fish growth, we compared growth rates of rainbow trout (*Oncorhynchus mykiss*) in nine closely located warm-temperate lakes of contrasting morphometry, stratification and mixing regime, and trophic state. Analyses of long-term mark-recapture data showed that in deep oligotrophic and mesotrophic lakes, trout growth rates increased with increasing indices of lake productivity. In contrast, in shallow eutrophic lakes, where fish habitat volume is constrained by temperature and dissolved oxygen, trout growth rates declined with increasing productivity. Growth rates were higher in lakes with greater volumes of favorable habitat (i.e., dissolved oxygen >6.0 mg/L and temperature <21°C) and lower in lakes with increased turbidity, chlorophyll a, and nitrogen concentrations. Our findings suggest that increases in lake productivity and temperatures as a result of global climatic change are likely to be more detrimental to salmonid habitat quality in shallower, productive lakes, while salmonids will better endure such changes in deeper, oligotrophic lakes. Fishery managers can use this information to aid future stocking decisions for salmonid fisheries in warm-temperate climates.

Blair et al. 2013b

1. To investigate the carrying capacity and factors affecting growth of rainbow trout in Lake Rotoiti, we employed a bioenergetics model to assess the influence of stocking rates, timing of releases and prey abundance on growth and prey consumption. We hypothesized that stocking rates and prey abundance would affect growth and prey consumption by influencing per-capita prey availability, and that the environmental conditions encountered by fish at the time of stocking would affect growth and consumption.
2. Prey consumption of stocked rainbow trout was calculated with the Wisconsin bioenergetics model. We calculated growth trajectories of released trout based on data from stocked trout that were released in spring and autumn from 1993 to 2009 and then re-captured by anglers. Diet, prey energy density, body mass lost during spawning and lake temperature were measured locally.
3. Stocking timing had no effect on return rates to anglers or length or weight of caught fish. Although trout released in autumn were smaller than those released in spring, autumn-released trout grew at a faster rate and had similar lengths and weights to spring cohorts after 2 years of growth in the lake. Modelled consumption parameters were negatively correlated with trout population size, suggesting that stocking rates (347 to 809 fish/ha in 1 year) caused density-dependent effects on growth. Although common smelt (*Retropinna retropinna*) accounted for 85 percent of total prey consumption, no significant relationship was found between prey consumption by individual trout and adult smelt abundance, possibly because trout are targeting smaller smelt that our abundance estimate did not account for.

4. Releasing trout in autumn appears to be advantageous for growth, possibly because
 - (i) temperature is more suitable for growth in autumn–winter than in spring–summer and
 - (ii) prey for small trout is abundant in autumn. Mild winter conditions appear to enhance overwinter survival and growth of rainbow trout in warm-temperate lakes compared to higher latitudes. This implies that moderately productive warm-temperate lake ecosystems are highly suitable for trout growth in winter, but less so in summer, when lake stratification and high nutrient levels may create conditions suitable for algal blooms and hypolimnetic deoxygenation. High growth rates of trout in warm-temperate lakes can therefore be supported by timing releases to coincide with favorable winter conditions.

Brittain 2015

Freshwater ecosystems are subject to a wide variety of stressors, which can have complex interactions and result in ecological surprises. Non-native fish introductions have drastically reduced the number of naturally fishless lakes and have resulted in cascading food web repercussions in aquatic and terrestrial habitats. Additional anthropogenic influences that result from increases in global airborne emissions also threaten wildlife habitat. Atmospheric nitrogen deposition has been recognized as an anthropogenic contributor to acidification and eutrophication of wilderness ecosystems. Planktonic communities have shown declines in response to predation and shifts in composition as a result of nutrient inputs and acidification, both of which are potential fates of nitrogen deposition. This study identified the response of zooplankton communities from two lakes (fish present vs. absent) in Mount Rainier National Park to manipulations simulating an episodic disturbance event in mesocosms. The experiment used a 2 x 2 factorial design with acid and nitrogen treatments. Treatments resulted in significantly elevated nitrogen and decreased pH conditions from control mesocosms over 42 days, indicating that the treatment effects were achieved. Results indicate that zooplankton communities from lakes with different food web structure respond differently to the singular effects of acid and nitrogen addition. Surprisingly, the interaction of the two stressors was related to increases in community metrics (e.g., abundance, biomass, body size, richness, and Shannon-Weiner diversity) for both lake types. This work can aid management decisions as agencies look to restore more aquatic montane habitats to their historical fishless states, and assess their abilities to recover and afford resistance to atmospheric pollution.

Cucherousset and Olden 2011

There is a long history of introduction of non-native fishes in fresh waters and the introduction rate has accelerated greatly over time. Although not all introduced fishes have appreciable effects on their new ecosystems, many exert significant ecological, evolutionary, and economic impacts. For researchers, managers, and policy makers interested in conserving freshwater diversity, understanding the magnitude and array of potential impacts of non-native fish species is of utmost importance. The present study provides an illustrative conspectus of the most recent literature reporting ecological impacts of non-native freshwater fishes from a wide range of species and geographic locations and concludes with a prospectus of needed areas of scientific inquiry. Both directly and indirectly, invasive fishes affect a wide range of native

organisms from zooplankton to mammals across multiple levels of biological organizations ranging from the genome to the ecosystem. Although a great deal of knowledge has been recently accumulated, this body of knowledge dwarfs in comparison to what we still need to learn. Specifically, we cite the need for additional scientific inquiry to fill knowledge gaps that are principally caused by taxonomically, geographically, disciplinarily, and methodologically unbalanced approaches.

Dunham et al. 2004

Intentional introductions of non-native trout into headwater lakes and streams can have numerous effects on the receiving ecosystems, potentially threatening native species and disrupting key ecological processes. In this perspective, we focus on seven key issues for assessing the biological and economic consequences of non-native trout in headwater ecosystems: (1) effects of non-native trout can span multiple biological domains, (2) effects of non-native trout can extend beyond waters where they are introduced, (3) non-native trout do not travel alone, (4) not all habitats are equal, (5) ecosystems vary in their resistance and resilience to non-native trout, (6) prioritization can improve management of non-native trout, and (7) economic costs of recreational fisheries in headwater ecosystems can be substantial. Assessments that address these issues could provide more effective guidance for determining where recreational fisheries for non-native trout are justified in headwater ecosystems and where they might be terminated to support other ecosystem values.

Eby et al. 2006

The establishment of exotic game fishes to enhance recreational fisheries through authorized and unauthorized stocking into freshwater systems is a global phenomenon. Stocked fishes are often top predators that either replace native top predators or increase the species richness of top predators. Many direct effects of stocking have been documented, but the ecosystem consequences are seldom quantified. New studies increasingly document how species and community shifts influence ecosystem processes. We discuss here how predator stocking might increase top-down effects, alter nutrient cycles and decrease links between aquatic and surrounding terrestrial ecosystems. As fisheries management moves beyond species-specific utilitarian objectives to incorporate ecosystem and conservation goals, ecologists must address how common management practices alter food-web structure and subsequent ecosystem-level effects.

Elser et al. 1995

Stocking of the dominant planktivore of Castle Lake (rainbow trout) was discontinued to examine the impact of food web interactions on zooplankton communities and inter- and intra-annual dynamics of ecosystem properties (light penetration, primary productivity). Dynamics of zooplankton and ecosystem processes were examined for 3 years following the manipulation and compared to 2 to 3 years of premanipulation data. Sampling of vertebrate and invertebrate

planktivores documented shifts in other members of the zoo-planktivore guild as rainbow trout declined. Reduction of rainbow trout densities led to compensatory responses in other components of the Castle Lake fish assemblage as brook trout and golden shiners increased in abundance. This compensation resulted in increased rates of vertebrate planktivory on daphnids within 2 years after trout stocking was discontinued. Zooplankton shifts in response to discontinuance of trout stocking were more rapid, particularly an immediate increase in a previously rare invertebrate predator (*Diacyclops thomasi*). Other limnological parameters also responded rapidly following the manipulation: water transparency declined and primary productivity (PPr) increased. In addition, intra-annual patterns (i.e., seasonal development) and the vertical distribution (shallow vs. deep) of PPr appeared to be affected by the food web manipulation. Our results indicate that complexities of real food webs complicate the prediction of the outcome of food web perturbations. Reduction of the previously dominant planktivore (rainbow trout) led to increases in other zooplanktivores (*Diacyclops*, golden shiners, brook trout) that resulted in enhanced predation pressure on zooplankton herbivores. Our results also indicate that alterations in water quality parameters (transparency, PPr) in response to food web alterations need not necessarily be mediated through changes in the abundance of *Daphnia*, as strong limnological responses preceded reductions in *Daphnia* by a year. We hypothesize an alternative mechanism for food-web-induced changes in lake ecosystem dynamics: changes in water clarity and productivity can result when cyclopoid predation strongly affects micrograzers.

Gozlan 2007

Risk perceptions are important to the policy process, but there is often a well-established pattern of small risks being over assessed. This is also true with the issue of non-native freshwater fish introductions, where a great majority of research focuses on the few negative cases. The attitude towards “non-natives” is a continually evolving process and varies according to current societal values. Here I show that on the global scale, the majority of freshwater fish introductions are not identified as having an ecological impact while having great societal benefits. Case studies from the African lakes are discussed in order to illustrate contrasting outcomes following fish introductions. Looking into the future, the environmental changes that freshwater ecosystems may encounter will have inevitable implications on the distribution of our native freshwater fish species and the need to rely on non-native introductions may become a growing reality. Aquaculture production is regularly increasing and our dependence on it is likely to become greater as it provides an important substitute for the declining production of capture fisheries. With it the number of freshwater fish introductions will increase and a more realistic attitude, albeit controversial, will need to be debated. This would mean protecting some introductions that present beneficial outcomes for biodiversity alongside a more systematic ban of species or families of fish presenting a higher historical ecological risk. The public perception of risk is something which cannot be ignored by any government or ruling body, but in order to gain public support in the fight for conservation of freshwater fish biodiversity, the message needs to be clear, detailed and educational.

Gunn et al. 1987

Juvenile, hatchery-reared lake trout (*Salvelinus namaycush*), 25 to 36 months old, were stocked (about 24 fish/hectare) in six small oligotrophic lakes to test the effects of fish size, stocking season, and lake water acidity on survival and growth of introduced fish. The test lakes had few, if any, native lake trout. Little or no survival of stocked lake trout occurred in lakes with pH 5.0 or less. High survival and growth occurred in intermediately acidic (pH 5.6 to 6.1) and circumneutral (pH 6.9 to 7.3) lakes. Survival of stocked lake trout increased with size at the time of stocking. Size differences of the three size-classes introduced in each lake were maintained throughout the 2-year study. Competition with other resident fish species appeared to influence stocking success strongly. There was an inverse relationship between the biomass of stocked lake trout subsequently recaptured and biomass of all hypolimnetic species present in the lake. Intraspecific competition with previously stocked lake trout also appeared to restrict survival of lake trout in subsequent stockings.

Hanisch et al. 2013

1. Rainbow trout (*Oncorhynchus mykiss* [Walbaum]) is commonly stocked as a sport fish throughout the world but can have serious negative effects on native species, especially in headwater systems. Productive fish-bearing lakes represent a frequently stocked yet infrequently studied system, and effects of trout in these systems may differ from those in headwater lakes.
2. We used a Before-After Control-Impact (BACI) design to determine how stocked trout affected assemblage-level and taxon-level biomass, abundance and average length of littoral invertebrates in a stocked lake relative to three unstocked control lakes in the boreal foothills of Alberta, Canada. Lakes were studied 1 year before and for 2 years after stocking. Because characteristics of productive fish-bearing lakes should buffer impacts of introduced fish, we predicted that trout would not affect assemblage-level structure of littoral invertebrates but might reduce the abundance or average length of large-bodied taxa frequently consumed by trout.
3. Relative to the unstocked control lakes, biomass, but not abundance, of the littoral invertebrate assemblage was affected indirectly by trout through increases of some taxa after trout stocking. At the individual taxon-level, trout stocking did not affect most (23 of the 27) taxa, with four taxa increasing in abundance or biomass after stocking. Only one taxon, Chironomidae, showed evidence of size-selective predation by trout, being consumed frequently by trout and decreasing significantly in average length after stocking.
4. Our results contrast with the strong negative effects of trout stocking on invertebrate assemblages commonly reported from headwater lakes. A combination of factors, including large and robust native populations of forage fish, the generalized diet of trout, overwinter aeration, relatively high productivity and dense macrophyte beds, likely works in concert to reduce potentially negative effects of stocked trout in these systems. As such, productive,

fish-bearing lakes may represent a suitable system for trout stocking, especially where native sport fish populations are lacking.

Hembre 2016

No abstract provided.

Holmes et al. 2017

Stocking lakes with trout can have strong effects on native communities; however, the nature of impacts is not universal across receiving ecosystems. To assess effects of non-native trout, relative to native small-bodied fish, on microcrustacean zooplankton, we compared stocked, unstocked (but fish-bearing), and fishless lakes in the boreal foothills of Alberta, Canada. Relative to unstocked lakes, stocked lakes had greater richness, but otherwise showed few additional effects on microcrustacean communities. In contrast, fishless lakes supported lower abundances of Cladocera, Calanoida, and Cyclopoida, but were dominated by larger cladoceran and calanoid taxa, compared with fish-bearing lakes (stocked and unstocked). Vertical distributions also differed significantly among lake types; microcrustaceans had far higher relative abundances at 1 m than at 2 m in fishless lakes compared with fish-bearing lakes (distributions in stocked and unstocked lakes were similar). Microcrustacean communities in fishless lakes were likely shaped by the invertebrate planktivore *Chaoborus*, which was abundant in these systems, whereas native fishes likely structured microcrustacean communities prior to trout introductions, with planktivory by trout causing few additional effects.

Koenig and Meyer 2011

Idaho Department of Fish and Game hatcheries stock predominantly sterile triploid (3n) rainbow trout *Oncorhynchus mykiss* to provide sportfishing opportunities while minimizing the genetic risks to wild stocks. Triploid catchable-sized rainbow trout are stocked in over 500 water bodies across Idaho annually, but there remains some uncertainty regarding the performance of triploid rainbow trout relative to their diploid (2n) counterparts. We examined the relative survival, growth, and returns of diploid and triploid all-female catchable rainbow trout across 13 lakes and reservoirs. Most reservoirs showed higher returns of 2n rainbow trout to anglers. In 2008, 3n rainbow trout returned on average at only 72 percent and 81 percent of the rates of 2n trout in gill nets and snout collection boxes, respectively, and the difference for both methods was statistically significant. Carryover of marked rainbow trout from 2008 was low or zero in most reservoirs. Where there was carryover, snout collection boxes suggested that 3n rainbow trout returned to anglers at 71 percent of the rate of 2n rainbow trout in the second year after planting, but the difference was not statistically significant. Triploid rainbow trout did not show any growth advantages over 2n rainbow trout but were similar in length, weight, and dressed weight. The disparity in returns between 2n and 3n trout varied across reservoirs but was more pronounced in locations subjected to greater drawdown and with greater species diversity. While 2n rainbow trout may grow and survive better in reservoirs subject to low water levels,

triploid rainbow trout may perform equally well under good habitat conditions while not having genetic impacts on native stocks. These findings are rather fortuitous for fisheries managers, as triploids probably perform better in higher quality habitats where native trout often exist, whereas diploids are better suited to reservoirs with degraded habitats where native stocks have usually been extirpated.

Knapp et al. 2005

The ratio of the number of taxa observed at a site to that expected to occur in the absence of anthropogenic impacts (O/E) is an ecologically meaningful measure of the degree of faunal alteration. We used O/E ratios to describe the response by amphibian, reptile, benthic macroinvertebrate, and zooplankton taxa in originally fishless lakes in Yosemite National Park to the introduction and subsequent disappearance of non-native fish. To quantify resistance (the degree to which a system is altered when the environment changes) and resilience (the degree to which a system returns to its previous configuration once the perturbation is removed), we compared O/E ratios between lakes that were never stocked, were previously stocked and still contained fish, or were previously stocked but had reverted to a fishless condition. On average, stocked-fish-present sites had 16 percent fewer taxa than never-stocked sites (O/E = 0.84 vs. 1.00, respectively). This statistically significant difference in O/E ratios indicates that native fauna had relatively low resistance to fish introductions. Resistance was inversely related to fish density and elevation, and directly related to water depth. Vulnerability to impacts of trout predation differed markedly between faunal groups, being high for amphibians, reptiles, conspicuous benthic invertebrates, and zooplankton and low for inconspicuous benthic invertebrates. O/E ratios in stocked-now-fishless sites were significantly higher (1.00) than those in stocked-fish-present sites and were not significantly different from those in never-stocked sites, indicating that this fauna had high resilience. For stocked-now-fishless sites, the relationship between the O/E ratio and the number of years since fish disappearance indicated that taxonomic composition recovered to closely resemble that of never-stocked lakes in less than 2 years following fish disappearance. Collectively, these results indicate that despite strong effects of an introduced predatory fish on community structure, these systems recover quickly and predictably following fish removal.

Lancelotti et al. 2017

1. Aquaculture in arid Patagonia is potentially affecting the hooded grebe (*Podiceps gallardoi*), a critically endangered endemic water bird. Exotic rainbow trout (*Oncorhynchus mykiss*) were stocked from 1994 in naturally fishless lakes, the primary reproductive habitat of this grebe.
2. Trout and grebes are visual predators, whose diets overlap. Consequently, trout could reduce the abundance of prey of the hooded grebe.
3. This study compared the size distribution and abundance of the pelagic zooplankton fraction preyed upon by trout in four fishless lakes and three lakes stocked with trout, including vegetated and unvegetated lakes.

4. The mean size of *Daphnia* spp. was 45 percent and 35 percent larger in fishless lakes than in stocked lakes, for unvegetated and vegetated lakes, respectively. *Boeckella* spp. were larger in fishless than in stocked vegetated lakes.
5. Fishless and stocked lakes had highly contrasting biomasses of large pelagic crustaceans. Amphipods were absent from the water column of all stocked lakes analyzed, and were abundant in fishless lakes. *Parabroteas sarsi* was absent from the two large unvegetated lakes, stocked with trout.
6. These shifts in the abundance and size spectrum of the zooplankton may reflect competition between trout and hooded grebe, affecting the survival of the latter species.
7. The current conservation status of this rare aquatic bird demands the application of management tools to reduce the detrimental effects of aquaculture on their primary reproductive habitat.

Losee and Phillips 2017

The effect of the length of rainbow trout (*Oncorhynchus mykiss*) on catch rate, catch size, and cost of stocking was evaluated in two western Washington lakes. Rainbow trout of two general length classes (200 to 300 [“catchables”] and 300 to 400 mm [“jumbos”]) were differentially marked and stocked 1 d prior to a fishery utilizing typical sportfishing techniques. In both study lakes, larger trout represented a larger proportion of the total catch than would have been expected if proportional to lengths at stocking. Fish in the largest individual length-class (360 to 380 mm) were, on average, 12.5 times more likely to be caught by sport anglers than those in the smallest individual length-class (200 to 220 mm). Larger rainbow trout are more expensive to produce for stocking agencies. However, fish stocked in the 280- to 300-mm class were twice as likely to return to the creel as expected and therefore are the best value in terms of number of fish caught per dollar invested. The results of this study suggest that a stocking strategy aimed at stocking fish 280 mm or larger would result in lower cost overall and increased satisfaction from anglers. Additionally, stocking rainbow trout less than 280 mm appears to have minimal recreational value in western Washington lakes

Lyons et al. 2016

In high-elevation lakes of the Sierra Nevada Mountains of central California, USA, increases in P concentration suggest accelerated nutrient loading in these delicate aquatic ecosystems. Some of these lakes show signs of eutrophication due to increased P loading. Presently, fish stocking practices include introductions of non-native as well as native fish, and sometimes in very large quantities. Stocked fish are fed diets that are often high in P and in turn excrete high P waste into lakes and/or die and decompose, potentially adding additional P to the system. The goal of this research was to determine the potential P contributions from residential shoreline developments and stocked fish. A seasonal, steady state P loading rate model was created to quantify P loading into nine lakes in the eastern Sierra Nevada. Lakes with no fish, stocked lakes,

and lakes that have shoreline developments and stocked fish were compared using measured P concentrations. The greatest difference in P-loading rates was between stocked and unstocked lakes, which yielded an average of 6.29×10^{-3} ($\pm 6.39 \times 10^{-3}$) mg P·L⁻¹·yr⁻¹ and 6.87 (± 5.41) mg P L⁻¹·yr⁻¹, respectively. Stocked lakes with shoreline development did not vary significantly from lakes with stocked fish and no shoreline development. The P-loading rate showed a correlation with the annual frequency of stocking events with an R^2 value of 0.73.

Martin et al. 2018

Flavobacterium psychrophilum is a pathogen causing bacterial Coldwater disease in salmonid hatcheries worldwide. This study evaluated the feeding of four commercial diets to rainbow trout *Oncorhynchus mykiss* at a hatchery with endemic bacterial Coldwater disease in three sequential feed trials, beginning at 28 days after initial feeding and lasting for a total duration of 83 days. Two Bio-Oregon (Longview, Washington, USA) diets, Bio-Vita and BioPro2 and two Skretting (Toele, Utah, USA) diets, Classic Trout and Protec, were used. While the overall mortality rates were the same among all the diets, there were differences in the timing at which the mortality occurred. Significant differences in tank ending weight, weight gain and feed conversion ratio only occurred in the third and final trial, with the Classic Trout diet producing the poorest results. Overall results, with all of the trials combined, indicated tanks receiving the Bio-Vita and BioPro2 diets had significantly higher weight gain than tanks receiving Classic Trout. Additionally, feed conversion ratios were significantly elevated in the Classic Trout treatment group compared to the other three dietary treatments. With few impacts on overall mortality among all diets tested, the faster growth obtained from the Bio-Oregon diets may lead to more rapid development of acquired immunity with possible implications for bacterial Coldwater disease management beyond the duration of this study.

Miko et al. 1995

By developing a winter fishery for rainbow trout *Oncorhynchus mykiss* to complement an existing warmwater sport fishery, fishery managers can promote higher angler use by providing year-round recreational fishing opportunities. However, the high cost of providing harvestable-size rainbow trout coupled with limited hatchery production capabilities and limited funds available for hatchery expansion and fishery programs will limit the expansion of these successful programs. We evaluated three stocking densities of harvestable-size rainbow trout—low (700 trout/ha), medium (1,400 trout/ha), and high (2,100 trout/ha)—to determine a stocking strategy that provides maximum fishery benefits from a limited number of fish. Catch rates were significantly higher for lakes with medium and high stocking densities. However, angler effort, proportion of stocked fish caught, angler fishing success rating, and angler trip satisfaction rating did not differ among stocking treatments. Despite catch rates of 0.5 trout/ha, anglers rated fishing success less than “fair” and trip satisfaction less than “good.” Before a stocking strategy can be designed, a management goal must be set because no single stocking strategy proved superior for all management goals considered.

Nasmith et al. 2012

Stocking lakes with trout for the purposes of recreational angling is a management strategy that introduces a new predator into these systems and thus deserves careful scrutiny. To assess the impact of non-native trout on littoral invertebrates in naturally fish-bearing lakes in the boreal foothills of Alberta, Canada, we compared their community composition, abundance, and size structure in stocked ($n = 5$) and unstocked ($n = 6$) lakes over a 2-year period. We detected no clear negative effects of introduced trout on invertebrate community composition and only few taxa-specific examples of decreased or increased invertebrate abundance. Furthermore, predation by trout had inconsistent direct effects on the size structure of invertebrate populations. Indirect effects were suggested by increased abundances and sizes of some invertebrate taxa in stocked lakes and might also contribute to the limited overall differences that we observed. We propose that net effects of stocked trout on littoral invertebrates are influenced by key characteristics of receiving ecosystems. In our boreal foothills lakes, dense macrophyte cover in warm littoral zones, high productivity, abundant forage fish, and limited densities of trout all likely combine to allow littoral invertebrate communities to withstand the impact of introduced trout with minimal effects.

Parker et al. 2001

Bighorn Lake, a fishless alpine lake, was stocked with non-native brook trout, *Salvelinus fontinalis*, in 1965 and 1966. The newly introduced trout rapidly eliminated the large crustaceans *Hesperodiaptomus arcticus* and *Daphnia middendorffiana* from the plankton. In July 1997, we began to remove the fish using gill nets. The population comprised 261 fish that averaged 214 g in wet weight and 273 mm in fork length. Thereafter, zooplankton abundance increased within weeks. Early increases were caused by the maturation of *Diacyclops bicuspidatus*, few of which reached copepodid stages before the removal of the fish because of fish predation. *Daphnia middendorffiana*, absent when fish were present, reappeared in 1998. *Hesperodiaptomus arcticus*, which had been eliminated by the stocked fish, did not return. The proportion of large zooplankton increased after fish removal, but their overall biomass did not change. Algal biomass was low and variable throughout the 1990s and correlated with water temperature but not with nutrient concentrations or grazer densities. Diatoms were the most abundant algal taxon in the lake, followed by Dinophyceae. Chrysophyceans and cryptophyceans were eliminated after the fish were removed. Chlorophyll *a* concentrations were unaffected. Gill netting is a viable fish eradication technique for smaller (less than 10 ha), shallow (less than 10 m deep) lakes that lack habitable inflows and outflows or other sensitive species. Further work is required to define appropriate removal methods for larger lakes and watersheds.

Redmond et al. 2018

Cumulative impacts of multiple stressors on freshwater biodiversity and ecosystem function likely increase with elevation, thereby possibly placing alpine communities at greatest risk. Here, consideration of species traits enables stressor effects on taxonomic composition to be translated into potential functional impacts. We analyzed data for 47 taxa across 137 mountain

lakes and ponds spanning large latitudinal (491 km) and elevational (1,399 m) gradients in western Canada, to assess regional and local factors of the taxonomic composition and functional structure of zooplankton communities. Multivariate community analyses revealed that small body size, clonal reproduction via parthenogenesis, and lack of pigmentation were species traits associated with both introduced non-native sportfish and also environmental conditions reflecting a warmer and drier climate—namely higher water temperatures, shallower water depths, and more chemically concentrated water. Thus, historical introductions of sportfish appear to have potentially induced greater tolerance in zooplankton communities of future climatic warming, especially in previously fishless alpine lakes. Although alpine lake communities occupied a relatively small functional space (i.e., low functional diversity), they were contained within the broader regional functional structure. Therefore, our findings point to the importance of dispersal by lower montane species to the future functional stability of alpine communities.

Schindler et al. 2001

The introduction of salmonid fishes into naturally fishless lakes represents one of the most prevalent environmental modifications of aquatic ecosystems in western North America. Introduced fish may alter lake nutrient cycles and primary production, but the magnitude and variation of these effects have not been fully explored. We used bioenergetics modeling to estimate the contributions of stocked trout to phosphorus (P) cycles across a wide range of fish densities in lakes of the Sierra Nevada, California. We also assessed the larger effects of fish-induced changes in phosphorus cycling on primary production using paleolimnological analyses from lakes in the southern Canadian Rockies. Our analyses showed that total P recycling by fish was independent of fish density but positively related to fish biomass in the Sierra Nevada. In lakes with fish populations maintained by continued stocking, fish recycled P at over twice the rate of those in lakes where introduced fish populations are maintained by natural reproduction and stocking has been discontinued. We estimate that P regeneration by introduced fishes is approximately equivalent to atmospheric P deposition to these lakes. Paleolimnological analyses indicated that algal production increased substantially following trout introductions to Rocky Mountain lakes and was maintained for the duration of fish presence. The results of our modeling and paleolimnological analyses indicate that introduced trout fundamentally alter nutrient cycles and stimulate primary production by accessing benthic P sources that are not normally available to pelagic communities in oligotrophic mountain lakes. These effects pose a difficult challenge for managers charged with balancing the demand for recreational fisheries with the need to maintain natural ecosystem processes.

Skinner et al. 2014

Line-diffuser hypolimnetic oxygenation (HO) was initiated in North Twin Lake, Washington, in 2009 to mitigate loss of coldwater fishery habitat due to temperature-dissolved oxygen “habitat squeeze” and to reduce internal phosphorus cycling. Active tracking, net-captures, and hydroacoustic analyses demonstrated that trout populations rapidly expanded into increased hypolimnetic habitat within the first few years of oxygenation; however, long-term fishery benefits and many basic ecological aspects of HO have yet to be established. Diet and food web

analyses indicate significant changes in feeding ecology of principal coldwater fish species in North Twin in 2012 compared to preoxygenation (2005) and to unoxygenated South Twin in 2012. North Twin rainbow trout (*Oncorhynchus mykiss*) consumed significantly more large-bodied *Daphnia* during midsummer 2012 than in South Twin, where rainbow trout fed primarily on littoral amphipods. Additionally, relative gut weight for brook trout (*Salvelinus fontinalis*) in August 2012 was significantly higher in North Twin compared to South Twin, apparently due to increased access to hypolimnetic zooplankton. Golden shiner (*Notemigonus crysoleucas*) diets also seem to include more zooplankton in oxygenated North Twin. Littoral-focused largemouth bass (*Micropterus salmoides*) diets were not altered by HO. Observed changes in feeding ecology following HO have significant implications for future fishery management in the Twin Lakes.

Tiberti et al. 2014a

Introduced fish seriously affect zooplankton communities in mountain lakes, often leading to the loss of large species. Selective predation is recognized to be the ultimate cause of such a strong impact. Here we describe the selection of zooplankton prey by analyzing the stomach contents of more than 300 brook trout (*Salvelinus fontinalis*) inhabiting seven alpine lakes in the Gran Paradiso National Park (western Italian Alps). Our results show that planktivory is much more common in young fish, which feed on a larger number of taxa, but also adult fish maintain the ability to feed on zooplankton. There is a direct dependence between the length of zooplankton prey and the length of their fish predators, and adult, not juvenile fish are responsible of the selective predation on large crustacean zooplankton, which drive the impact of introduced fish throughout the entire zooplankton community. In some rare cases, large zooplankton populations develop in the presence of brook trout, and planktivory can become an important temporary resource for adult fish during the ice-free season. Thus, in the early stages of the establishment of non-native trout in alpine lakes, large-bodied zooplankton may represent an important food resource.

Tiberti et al. 2014b

Fish introduction is a major threat to alpine lake biota leading to the loss of native species and to the degeneration of natural food-webs. This study provides an extensive investigation on the impact of the introduced fish *Salvelinus fontinalis* on the native communities of alpine lakes in the Gran Paradiso National Park. We compared the macroinvertebrate and zooplankton communities of six stocked and nine fishless lakes with a repeated sampling approach during the summers 2006 through 2009. The impact of fish presence on alpine lake fauna is often mediated by the strong seasonality governing these ecosystems, and it dramatically affects the faunal assemblage of littoral macroinvertebrates and the size, structure, and composition of the pelagic zooplankton community with a strong selective predation of the more visible taxa. Direct ecological impacts include a decrease or extinction of nonburrower macroinvertebrates and of large zooplankton species, while small zooplankton species and burrower macroinvertebrates were indirectly advantaged by fish presence. Due to the existence of a compensation between rotifers and crustaceans, fish presence does not affect total zooplankton biomass and diversity

even if fish are a factor of ecological exclusion for large crustaceans. These compensatory mechanisms are a key process surrounding the impact of introduced fish in alpine lakes.

Wissinger et al. 2006

1. Brown and rainbow trout have been introduced to many inland waters in New Zealand, but research on the impacts on native communities has focused mainly on streams. The purpose of this study was to compare the benthic communities of trout and troutless lakes. Based on previous studies in North America and Europe, we predicted that the benthic biomass, and especially the abundance of large invertebrates, would be lower in lakes with trout as compared to those without. We surveyed the invertebrate fauna of 43 shallow, high-elevation lakes (26 with and 17 without trout) in four geographic clusters on the central South Island and then conducted a detailed quantitative study of invertebrate biomass and community structure in 12 of these lakes.
2. Benthic community composition and diversity of lakes with and without trout were nearly identical and biomass was as high or higher in the lakes with as without trout. There was no evidence that trout have caused local extinctions of benthic invertebrates. Although the proportional abundance of large-bodied aquatic was slightly lower in lakes with than without trout, the abundance of several groups of large-bodied benthic taxa (dragonflies, caddisflies and water bugs) did not differ.
3. Our findings are in contrast to those in North American and Europe where trout introductions into previously troutless lakes have led to declines in the abundance of benthic invertebrates, especially large-bodied taxa. We propose that the modest effects of trout in New Zealand could be explained by (i) the high areal extent of submergent vegetation that acts as a benthic refuge, (ii) low intensity of trout predation on benthic communities and/or (iii) characteristics of the benthic invertebrates that make them relatively invulnerable to fish predation.
4. Regardless of the relative importance of these hypotheses, our results emphasize that the same invertebrates occurred in all of the lakes, regardless of size, elevation and presence of trout, suggesting habitat generalists dominate the benthic fauna in shallow New Zealand lakes.

Yule et al. 2000

We evaluated stockings of rainbow trout (*Oncorhynchus mykiss*) in Pathfinder and Alcova reservoirs, Wyoming, to determine what combination of strain, season of stocking, and size at stocking maximized angler catch in the presence of walleyes *Stizostedion vitreum*. Coded wire tags were used to identify individual rainbow trout to stock group. Angler catch of Kamloops rainbow trout and fall rainbow trout in Pathfinder Reservoir exceeded returns of Eagle Lake rainbow trout. Differences in strain performance in Alcova Reservoir were less pronounced. The importance of season of stocking was identified with fall-stocked (August through October)

rainbow trout returning to anglers in higher numbers than those stocked during spring (March through June). Size-at-stocking evaluations indicate that large, catchable-size (>208 mm total length) rainbow trout maximize use of hatchery facilities over stocking greater numbers of small, catchable (178 to 207 mm) or subcatchable (127 to 177 mm) sizes. Pond feeding trials conducted with three walleye size-classes and three rainbow trout sizes showed that 127-mm rainbow trout were highly vulnerable to walleyes as small as 330 to 378 mm. Intermediate-size rainbow trout (178 mm) were not readily consumed by 381 to 432-mm walleyes, but they were vulnerable to 483 to 533-mm walleyes. At 229 mm, rainbow trout appeared invulnerable to walleyes in the largest size-class (483 to 533 mm) we studied. Rainbow trout stocked at large, catchable sizes are probably vulnerable to fewer walleyes compared with small, catchable and subcatchable sizes, allowing greater numbers to survive predation and recruit to the sport fishery.

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APPENDIX B

Data Analysis Plan

TECHNICAL MEMORANDUM

Date: November 29, 2018

To: BiJay Adams and Jeremy Jenkins, Liberty Lake Sewer and Water District

Copy to: Lizbeth Seebacher, Washington Department of Ecology; Randall Osborne and Justin Spinelli, Washington Department of Fish and Wildlife; Joan Hardy, Washington Department of Health (retired); Daniel Schindler and Mike Brett, University of Washington; Tom Brattebo, Liberty Lake Resident; Chris Knutson, King County Water and Land Resources Division; Isabel Ragland, Pierce Conservation District; Marisa Burghdoff, Snohomish County Surface Water Management; and Jane Mountjoy-Venning, Thurston County Public Health and Social Services

From: Rob Zisette, Herrera Environmental Consultants

Subject: Data Analysis Plan for the Trophic Cascade Effects on Algae Blooms Project

INTRODUCTION

This memorandum presents a summary of the available data and initial plans for analysis of the data for the Trophic Cascade Effects on Algae Blooms in Washington Lakes Project. This memorandum is distributed to the project technical committee for discussion at the project kickoff meeting on December 3, 2018. The data analysis plan may be modified based on input from the technical committee and further investigation of the available data.

The study hypotheses include:

- Algae blooms of primary concern are those comprised of cyanobacteria, which include some species that produce various cyanotoxins at unpredictable rates.
- Cyanobacteria blooms are primarily driven by the phosphorus supply (i.e., bottom up control).
- Cyanobacteria blooms may be stimulated on occasion by the nitrogen supply for some species that can use atmospheric nitrogen when nitrate and ammonia concentrations are low.
- Cyanobacteria blooms also may be stimulated in part by climatic factors including high water temperatures and low wind mixing that favor cyanobacteria growth over other types of algae.



- Cyanobacteria blooms may be reduced by zooplankton grazing (i.e., top down control), primarily by Daphnia and other large cladocerans that can consume small to moderately-sized cyanobacteria colonies.
- Cyanobacteria blooms may be stimulated by preferential grazing of zooplankton on other types of more edible algae.
- Cladoceran populations primarily increase in response to algae growth as their primary food source.
- Cladoceran populations decrease from predation by small fish, which primarily include small warm-water fish and trout up to legal size in Washington lakes, but may also decrease from predation by predatory zooplankton species such as Chaoborus.
- Stocking lakes with small trout may decrease cladoceran populations and stimulate cyanobacteria blooms.
- Stocking lakes with large trout and other fish that prey on small fish may increase cladoceran populations and reduce cyanobacteria blooms.

LIBERTY LAKE DATA ANALYSIS

Data available for the Liberty Lake data analysis are summarized in Table 1. Water quality data are available from April through October in all years for all field parameters since 1983 and all laboratory parameters interest since 1974 except for some gaps for total nitrogen and larger gaps for chlorophyll. Chlorophyll is only available for 8 years prior to 2012 and has since been collected consistently using a probe at 1-meter depth intervals. Water quality data are consistently available for three depths (Surface/Mid-depth/Bottom) at two lake stations named Northwest and Southwest.

Phytoplankton data as cell number and biovolume for eight groups (phyla) have been collected consistently since 2000 at the three depths and two lake stations. Data before 2000 include 8 years of cell number data and 3 years of biovolume data that do not coincide. Zooplankton data have been collected using vertical tows at both stations consistently since 1983 and are compiled for five groups (classes). Cyanotoxin data are only available for a few shore scum samples collected in each of 4 years (2008, 2009, 2015, and 2016).

Fish stocking data are available and complete since 1967. These data include the numbers and biomass (pounds) of each stocked species and size class of fish. Most of the fish were planted in the lake during spring season (April through June) and many were also planted in the late summer/fall season (August through October).

Fish stocking data are presented for each year since 1995 for 3 fish species (rainbow trout, brown trout, and walleye) and two life state stages (fry and legal-size) as numbers in Figure 1

and as mass (pounds) in Figure 2. For simplification, some rarely stocked species were included in one of these fish species categories (i.e., rainbow fry include one stocking of steelhead fingerlings, brown trout include a few stockings of eastern brook trout and tiger trout, and walleye includes a few stockings of sauger/walleye). These graphs show that Liberty Lake has been stocked by a wide variety of species and size classes throughout the years, with all species of fry dominating the numbers stocked on different years, and the total biomass stocked is typically dominated by legal-size rainbow trout followed by legal-size brown trout.

An analysis plan for Liberty Lake data is presented in Table 2. Mean values of select water quality and plankton parameters will be calculated for the spring season (April through June), late summer/fall season (August through October), and the entire summer season used for trophic indices (May through October). Mean values will be calculated separate for each of the two stations, resulting in 6 station-season groups. Mean values will be calculated separately for various depths depending on the parameter that include surface (S), bottom (B), and/or all three depths (SMB). Maximum chlorophyll *a* values will be calculated for surface samples. Fish stocking numbers and biomass will be totaled for each season by species (RT, BT, and WE) and size class (fry and legal). This plan results in 70 metrics that include 21 water quality metrics, 27 phytoplankton metrics, 5 zooplankton metrics, 2 cyanotoxin metrics, and 15 fish stocking metrics.

Principal component analysis (PCA) will be used to evaluate all or a subset of the 70 metrics for each of six station-season groups of means (see Table 2). Each PCA plot will be examined to detect relationships among the metrics, and the primary metrics responsible for most of the variance (principal components) across the six station-season groups will be identified. Additional statistical analysis will be performed based on the PCA results. This analysis may include either multiple regression or decision tree analysis.

WASHINGTON LAKES DATA ANALYSIS

Table 3 presents a preliminary list of 98 lakes that have at least 10 years of water quality data for the Washington lakes data analysis. Data will be compiled from each source to determine the final list of lakes and the number of lake stations based on data completeness.

The second page of Table 3 includes the water quality parameters and sampling frequency typically used by each data source (county). The monitoring programs are similar among each source. For consistency among all Washington lakes, the parameters and depths monitored by Snohomish County will be used to define water quality metrics that are similar but less than those used for Liberty Lake. Plankton data will not be compiled because they are not consistently available for most of the Washington lakes. Cyanotoxin and dominant cyanobacteria species data will be compiled from the Washington State Toxic Algae website. Fish stocking data will be compiled from Washington State Department of Fish and Wildlife.

Mean values of nine water quality metrics and three cyanotoxin metrics will be calculated for each of three seasons (spring, late summer/fall, and entire summer). Fish stocking numbers and biomass will be totaled for each season for rainbow trout fry and legal-size, and will include total rainbow trout mass for a total of five fish metrics. Additional fish metrics may be added upon review of the available data and results of the Liberty Lake data analysis.

Principal component analysis (PCA) will be used to evaluate all 17 metrics for all three season groups (see Table 3) for each lake. The PCA plot of each lake will be examined to detect relationships among the metrics, and the primary metrics responsible for most of the variance (principal components) across the 98 lakes will be identified. Data for those primary metrics will be combined with trout stocking data normalized for lake area and possibly other lake attributes for PCA analysis of all lakes together to detect relationships among the types of study lakes. Additional statistical analysis will be performed based on the PCA results. This analysis may include either multiple regression or decision tree analysis.

TABLES

Table 1. Liberty Lake Data Available for the Trophic Cascade Project.

Table 2. Liberty Lake Data Analysis Plan for Trophic Cascade Project.

Table 3. Washington Lakes Selected for the Trophic Cascade Project.

Table 4. Washington Lakes Data Analysis Plan for the Trophic Cascade Project.

FIGURES

Figure 1. Annual Fish Stocking Numbers for Liberty Lake Since 1995.

Figure 2. Annual Fish Stocking Biomass for Liberty Lake Since 1995.

Table 1. Liberty Lake Data Available for the Trophic Cascade Project.

Parameter	Period of Record	Typical Locations	Typical Frequency	Data Gaps
Lake Water Quality				
Secchi Depth (m)	1982-2017	2 (NW/SW)	1-2/month, April-Oct	Minor to none
Temp (C)	1983-2017	2 (NW/SW)	1-2/month, April-Oct, 1m profile	Minor to none
DO (mg/l)	1983-2017	2 (NW/SW)	1-2/month, April-Oct, 1m profile	Minor to none
pH	1983-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
Cond (µs/cm)	1983-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
TP (mg/l)	1974-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
OP (mg/l)	1974-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
TN (mg/l)	1974-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Some missing or TKN data
NO2+NO3 (mg/l)	1974-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
NH3 (mg/l)	1974-2017	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	Minor to none
Chlor A (µg/l)	2013-2017	2 (NW/SW)	1-2/month, April-Oct, 1m profile	+ depths in 83-85, 06-08, 10-11
Phytoplankton (8 groups)				
Phytoplankton (Density - #/mL)	75-77, 85- 87, 90- 91, 00-17	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	78-84, 88-89, 92-99
Phytoplankton (Biovolume - µm/ml3)	83-84, 88, 00-17	2 (NW/SW)	1-2/month, April-Oct, S/M/B depths	75-82, 85-87, 89-99
Zooplankton (4 groups)				
Zooplankton (Density - # Zoo/m3)	1983-2017	2 (NW/SW)	1-2/month, April-Oct, Vertical tow	
Cyanobacteria Toxicity				
Microcystin (ug/L)	08-09, 15-16	Beaches	2-7/year total	Many
Anatoxin A (ug/L)	08-09, 15-16	Beaches	2-7/year total	Many
Fish Stocking				
Rainbow Trout Number/Pounds	1967-2018	One	3-7/yr in Spring/Fall, Fry and Legals	None (Smolt/Non-smolt pre-95)
Brook Trout Number/Pounds	1987-2018	One	2-3/yr in Spring/Fall, Fry and Legals	None (Smolt/Non-smolt pre-95)
Walleye Number/Pounds	1996-2018	One	0-2/yr in Spring/Fall, Fry and Legals	None (Smolt/Non-smolt pre-95)

Table 2. Liberty Lake Data Analysis Plan for Trophic Cascade Project.

Lake Water Quality Means	Phytoplankton Means ^a	Zooplankton Means ^a	Data Analysis
Secchi (m)	ChloroDen-S (cell/mL)	Cladocera (#/m3)	1. Principle Component Analysis of Station-Season Means/Totals NW-Spring (April-June) NW-Summer (May-Oct) NW-Fall (Aug-Oct) SW-Spring (April-June) SW-Summer (May-Oct) SW-Fall (Aug-Oct)
Temp-S (C)	ChloroBio-S (u3/mL3)	Daphnia (#/m3)	
TempDiff-S-B (C)	ChloroBio-SMB (u3/mL3)	Daphnia-Max (#/m3)	
DO-S (mg/l)	EuglenoDen-S (cell/mL)	Copepoda (#/m3)	
pH-S	EuglenoBio-S (u3/mL3)	Nauplii (#/m3)	
Cond-S (µs/cm)	EuglenoBio-SMB (u3/mL3)	Rotifera (#/m3)	
TP-S (mg/L)	PyrrhoDen-S (cell/mL)		
TP-B (mg/L)	PyrrhoBio-S (u3/mL3)	Cyanotoxin Means^a	
TP-SMB (mg/L)	PyrrhoBio-SMB (u3/mL3)	Microcystin (ug/L)	
OP-S (mg/L)	ChrysoDen-S (cell/mL)	Anatoxin (ug/L)	
OP-B (mg/L)	ChrysoBio-S (u3/mL3)		2. Multiple Regression of Decision Tree Analysis of Primary Metrics
TN-S (mg/L)	ChrysoBio-SMB (u3/mL3)	Fish Stocking Totals	
TN-B (mg/L)	BacillarioDen-S (cell/mL)	RTFry-num (#)	
NO2+NO3-S (mg/L)	BacillarioBio-S (u3/mL3)	RTFry-mass (lbs)	
NO2+NO3-B (mg/L)	BacillarioBio-SMB (u3/mL3)	RTLLegal-num (#)	
NH3-S (mg/L)	CyanoDen-S (cell/mL)	RTLLegal-mass (lbs)	
NH3-B (mg/L)	CyanoBio-S (u3/mL3)	RTTotal-mass (lbs)	
ChlorA-S (µg/L)	CyanoBio-S-Max (u3/mL3)	BTfry-num (#)	
ChlorA-S-Max (µg/L)	CyanoBio-SMB (u3/mL3)	BTfry-mass (lbs)	
ChlorA-B (µg/L)	CryptoDen-S (cell/mL)	BTLegal-num (#)	
ChlorA-SMB (µg/L)	CryptoBio-S (u3/mL3)	BTLegal-mass (lbs)	
	CryptoBio-SMB (u3/mL3)	BTTTotal-mass (lbs)	
	MicroDen-S (cell/mL)	WEFry-num (#)	
	MicroBio-S (u3/mL3)	WEFry-mass (lbs)	
	MicroBio-SMB (u3/mL3)	WELegal-num (#)	
	NannoDen-S (cell/mL)	WELegal-mass (lbs)	
	NannoBio-S (u3/mL3)	WETotal-mass (lbs)	
	NannoBio-SMB (u3/mL3)		

^a All values are mean values unless denoted by "Max". Use zero for phytoplankton/zooplankton groups without data.

All data from 1983 through 2018

Table 3. Washington Lakes Selected for the Trophic Cascade Project.

	SNOHOMISH COUNTY	KING COUNTY	PIERCE COUNTY	THURSTON COUNTY	EASTERN WASHINGTON
Lakes Total = 98	39	35	6	13	5
	ARMSTRONG	Alice	American	Barnes	Liberty
	BEECHER	Allen	Bonney	Black	Newman
	BLACKMANS	Ames	Debra Jane	Capitol	Twin East
	BOSWORTH	Angle	Gravelly	Clear	Twin West
	BRYANT	Beaver-1	Louise	Deep	Moses
	CASSIDY	Beaver -2	Steilacoom	Hicks	
	CHAIN	Bitter		Lawrence (2)	
	COCHRAN	Boren		Long (2)	
	CRABAPPLE	Cottage		Pattison North	
	CRYSTAL	Desire		Pattison South	
	ECHO	Dolloff		St Clair (2)	
	FLOWING	Echo		Summit	
	GISSBERG N.	Fivemile		Ward	
	GISSBERG S.	Forbes			
	GOODWIN	Geneva			
	HOWARD	Green			
	KAYAK	Joy			
	KETCHUM	Kathleen			
	KI	Killarney			
	LITTLE MARTHA	Langlois			
	LOMA	Lucerne			
	LOST	Marcel			
	MARTHA N.	Margaret			
	MARTHA S.	McDonald			
	MEADOW	Morton			
	NINA	Neilson			
	PANTHER	Paradise			
	RILEY	Pine			
	ROESIGER	Pipe			
	ROWLAND	Retreat			
	RUGGS	Sawyer			
	SERENE	Shadow			
	SHOECRAFT	Shady			
	SPRING	Spring			
	STEVENS	Twelve			
	STICKNEY				
	STORM				
	SUNDAY				
	WAGNER				

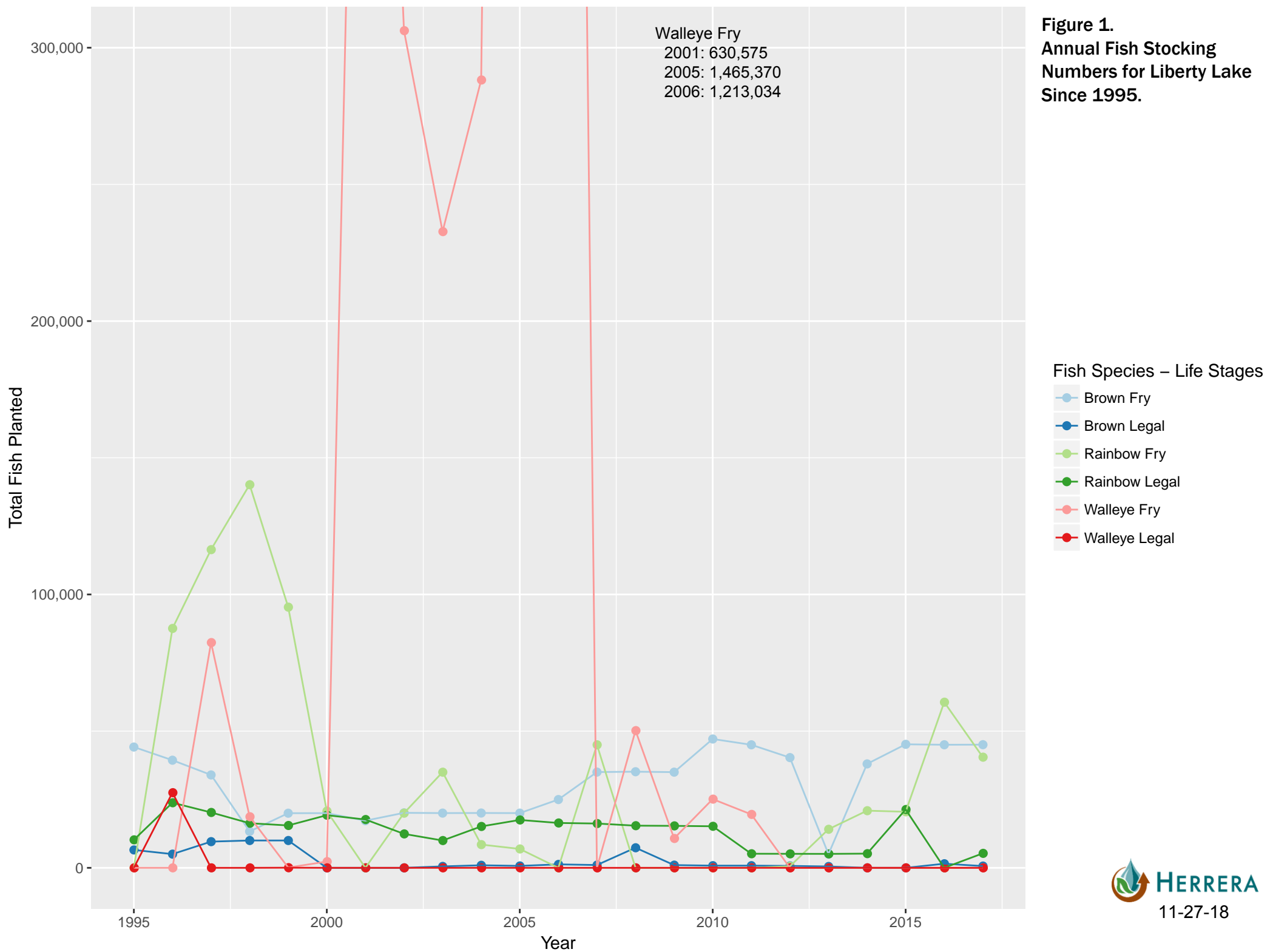
(2) = 2 stations on one lake

Table 4. Washington Lakes Data Analysis Plan for the Trophic Cascade Project.

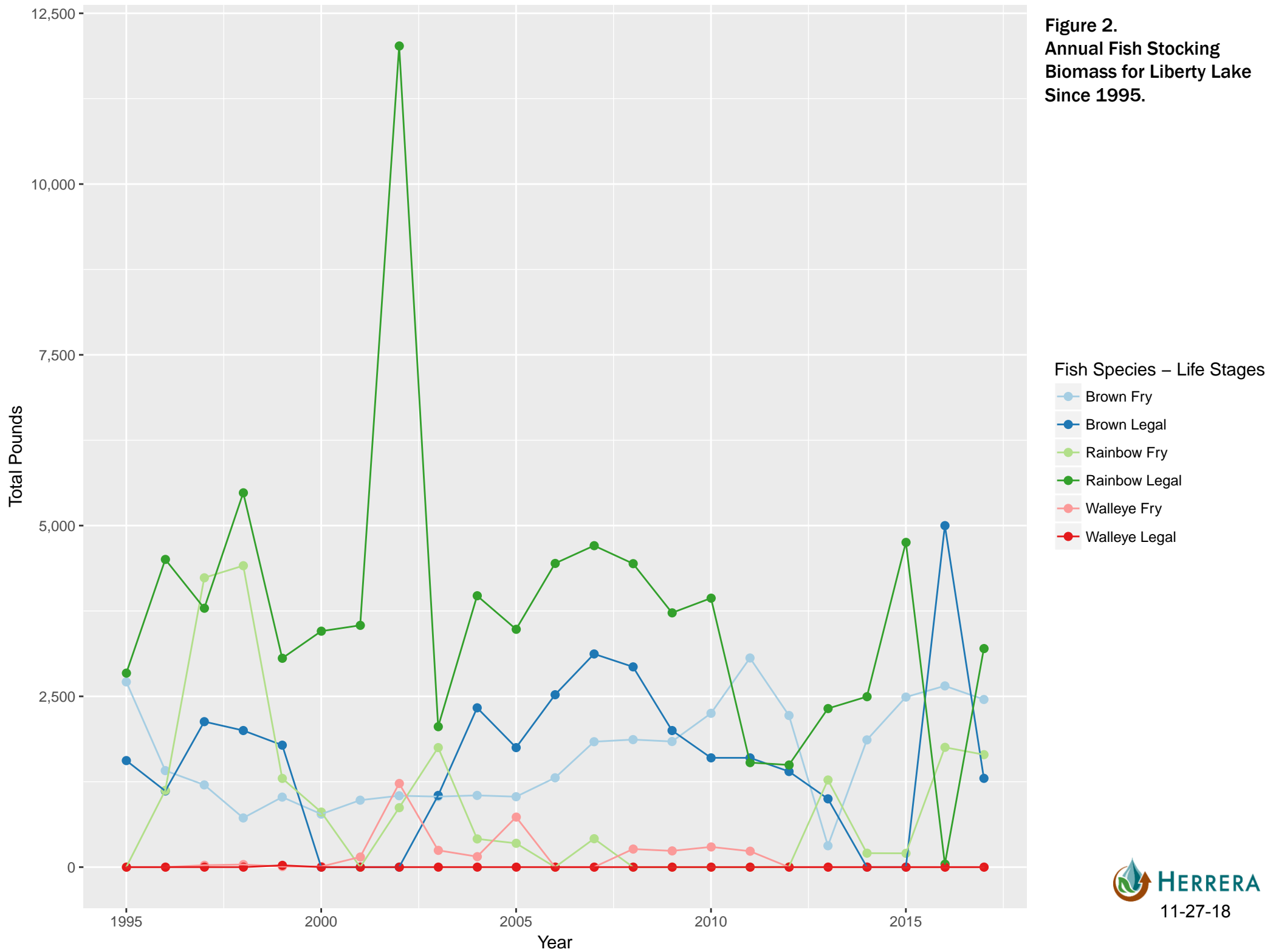
	SNOHOMISH COUNTY (39)	KING COUNTY (35)	PIERCE COUNTY (6)	THURSTON COUNTY (13)	EASTERN WASH (5)
WQ Parameters					
Secchi Depth (m)	S	S	S	S	S
Temp (C)	1m Profile	S,M,B	1m Profile	1m Profile	1m Profile
DO (mg/L)	1m Profile	None	1m Profile	1m Profile	1m Profile
pH	None	None	None	1m Profile	S,M,B
Cond (µs/cm)	None	None	None	1m Profile	S,M,B
TP (mg/l)	S,B	S,M,B	S,B	S,B	S,M,B
OP (mg/l)	None	S,B @ 2/yr	None	None	S,M,B
TN (mg/l)	S	S,M,B	S,B	S,B	S,M,B
NO2+NO3 (mg/l)	None	S,B @ 2/yr	Some S,B	None	S,M,B
NH3 (mg/l)	None	S,B @ 2/yr	Some S,B	None	S,M,B
Chlor A (µg/l)	S	S,M	S	S	S,M,B
Pheo A (ug/L)	S	S,M	S	S	S,M,B
WQ Frequency					
Start Year	1996+	1986+	2000+	1983+	1975+
Period	May-Oct	May-Oct	May-Oct	May-Oct	April-Oct
Frequency-Surface	2/mo	2/mo @ 1m	1/mo	1/mo	1-2/mo
Frequency-Subsurface	2/mo	2/yr @ M,B	1/mo	1/mo	1-2/mo
Phytoplankton					
Dominant Group	To be determined				
Cyanotoxins					
Scum Microcystin (ug/L)	Compiled from Washington State Toxic Algae Website for shore scum samples collected sporadically since 2007				
Scum Anatoxin A (ug/L)					
Scum Dominant Cyano					
Data Analysis					
Water Quality Means^a	Cyanotoxin Means^a	PCA of Each Lake by Season			
Secchi (m)	Microcystin (ug/L)	Spring (May-June)			
Temp-S (C)	Anatoxin (ug/L)	Summer (May-Oct)			
TempDiff-S-B (C)	CyanonDom (species)	Fall (Aug-Oct)			
DO-S (mg/l)					
TP-S (mg/L)	Fish Stocking Totals	PCA of all Lakes by Season			
TP-B (mg/L)	RTFry-num (#)	Spring (May-June)			
TN-S (mg/L)	RTFry-mass (lbs)	Summer (May-Oct)			
ChlorA-S (µg/L)	RTLegal-num (#)	Fall (Aug-Oct)			
ChlorA-S-Max (µg/L)	RTLegal-mass (lbs)	RTTotal-mass/acre (lbs)			
	RTTotal-mass (lbs)				

^a All values are mean values unless denoted by "Max".

Fish Stocking for Liberty Lake



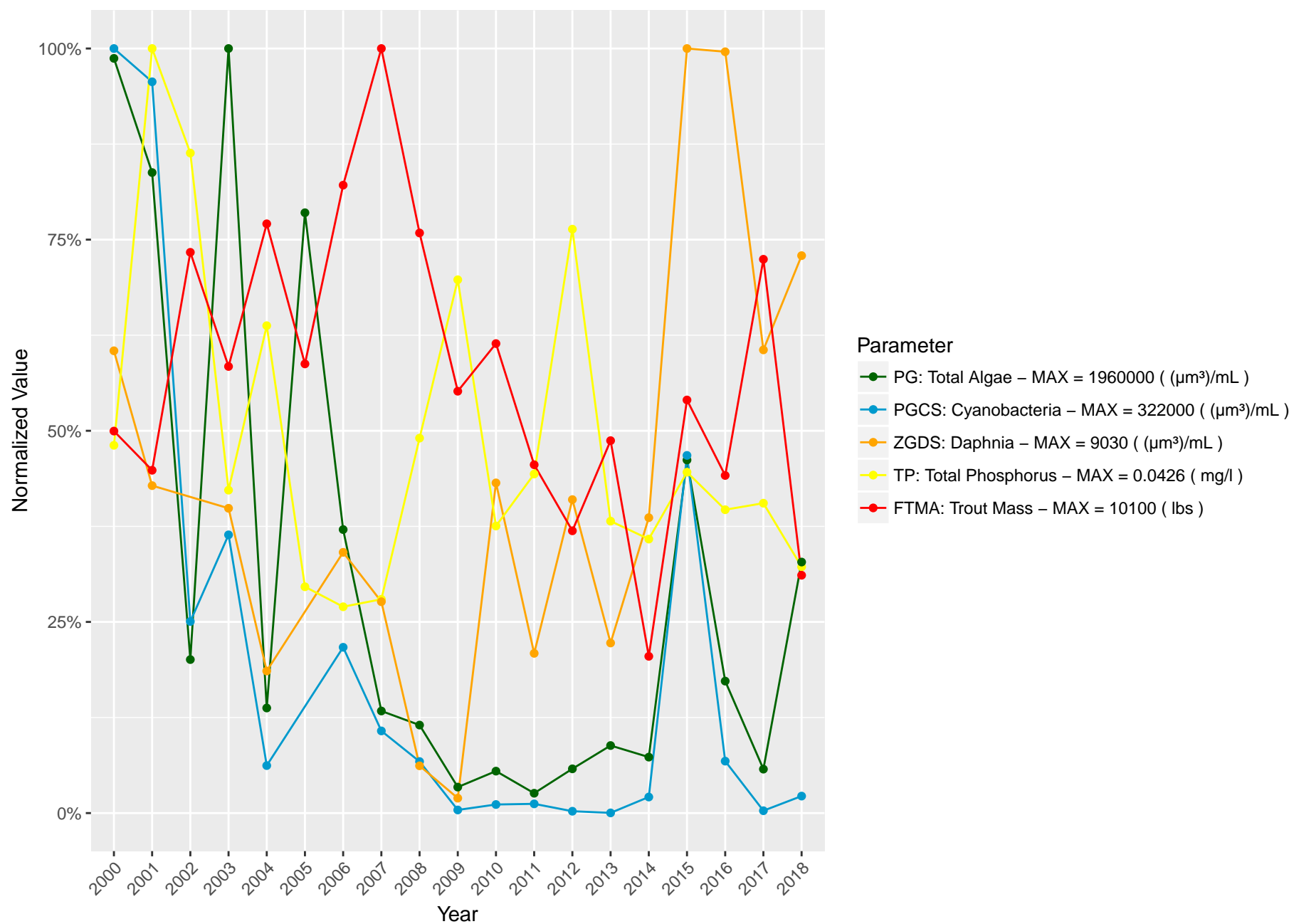
Fish Stocking for Liberty Lake



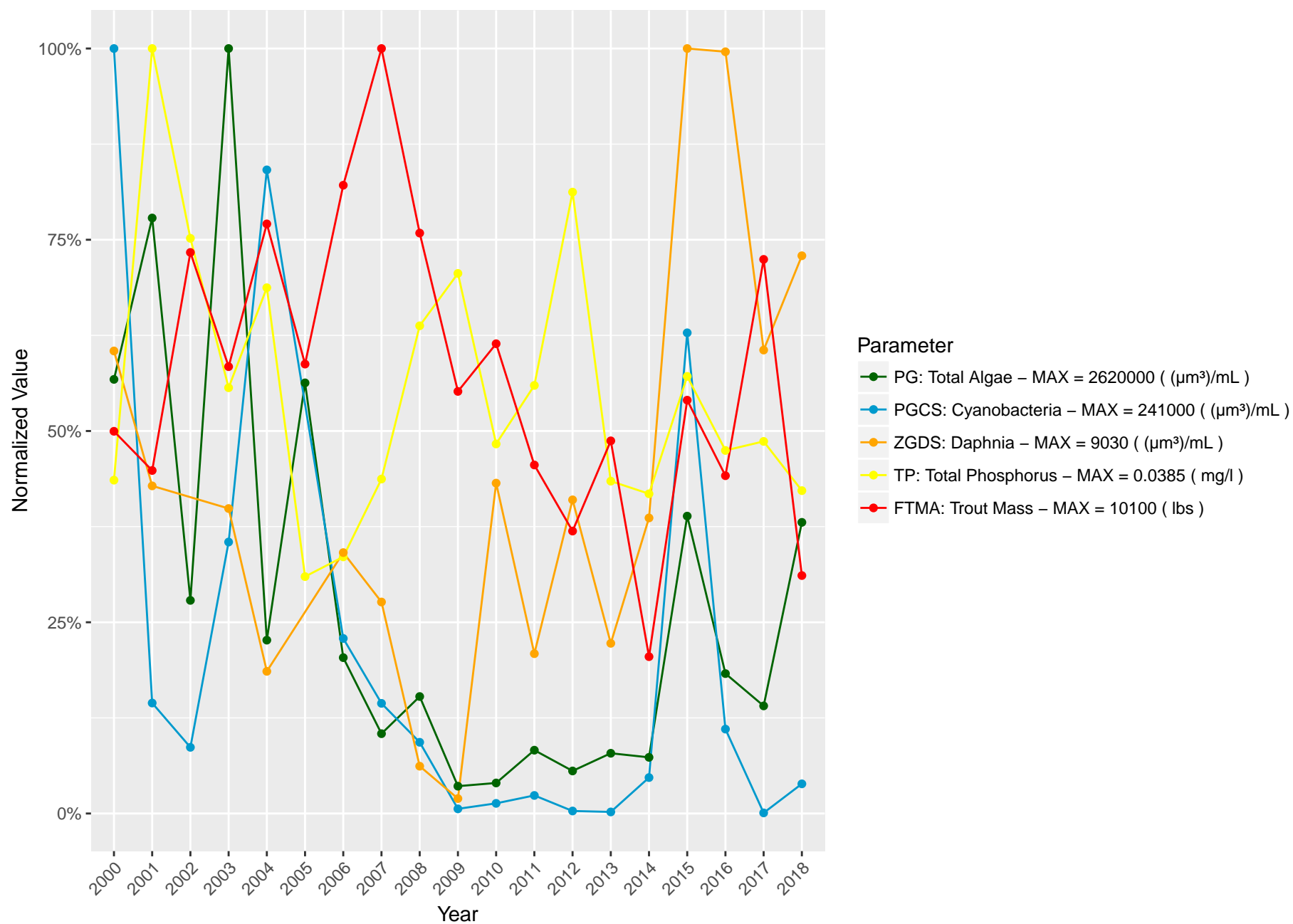
APPENDIX C

Liberty Lake Figures

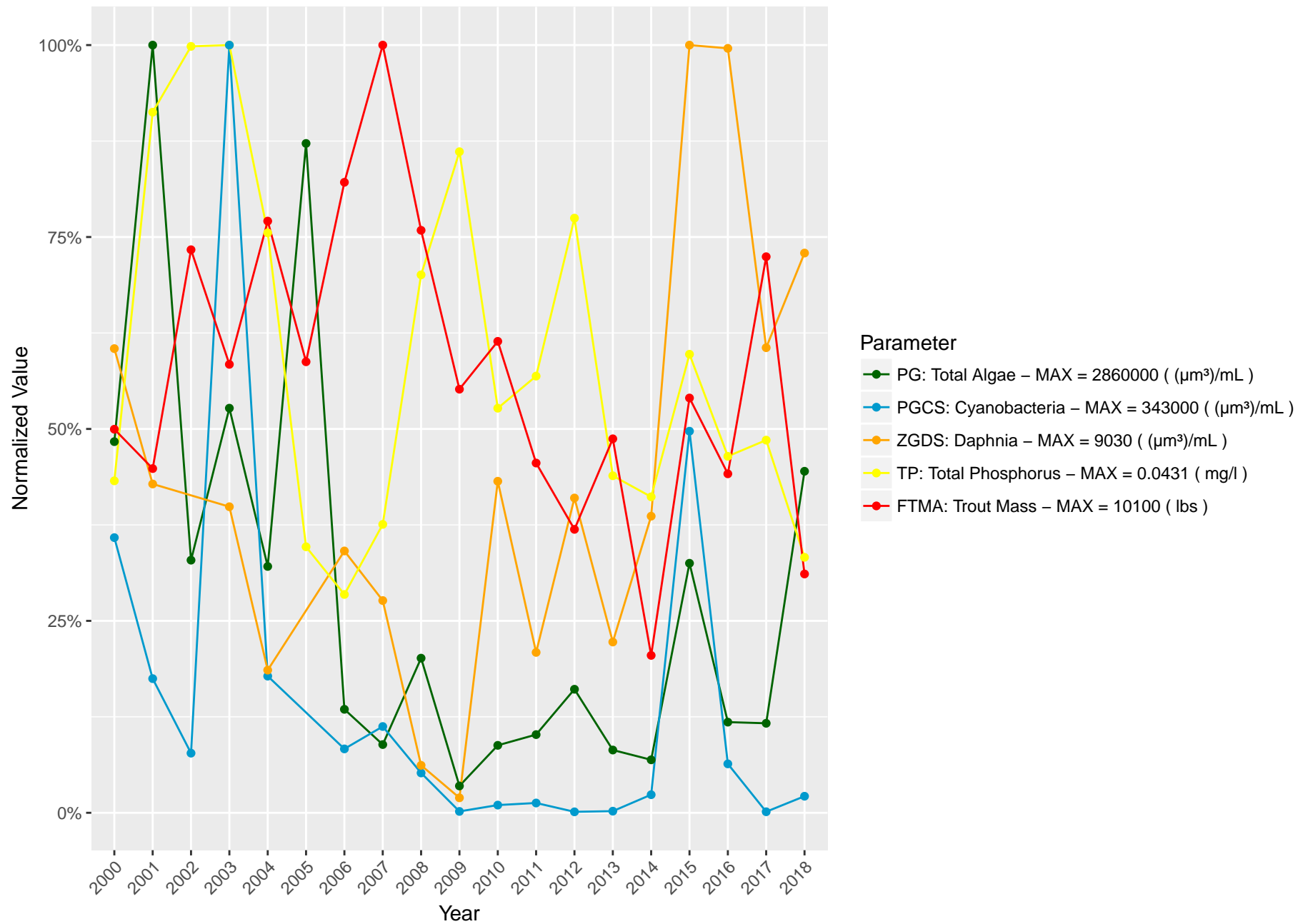
Liberty Lake – NWTOP



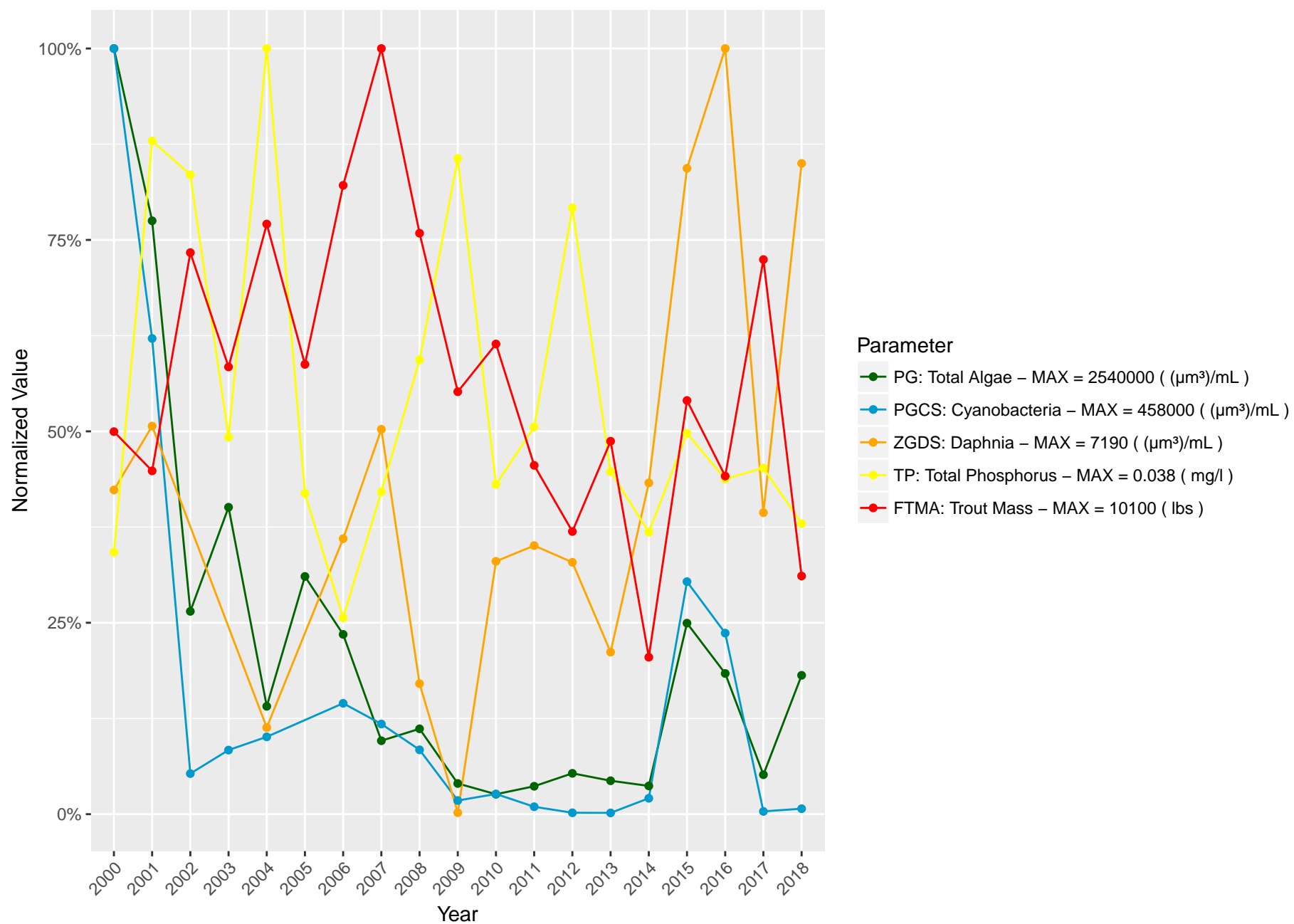
Liberty Lake – NWMID



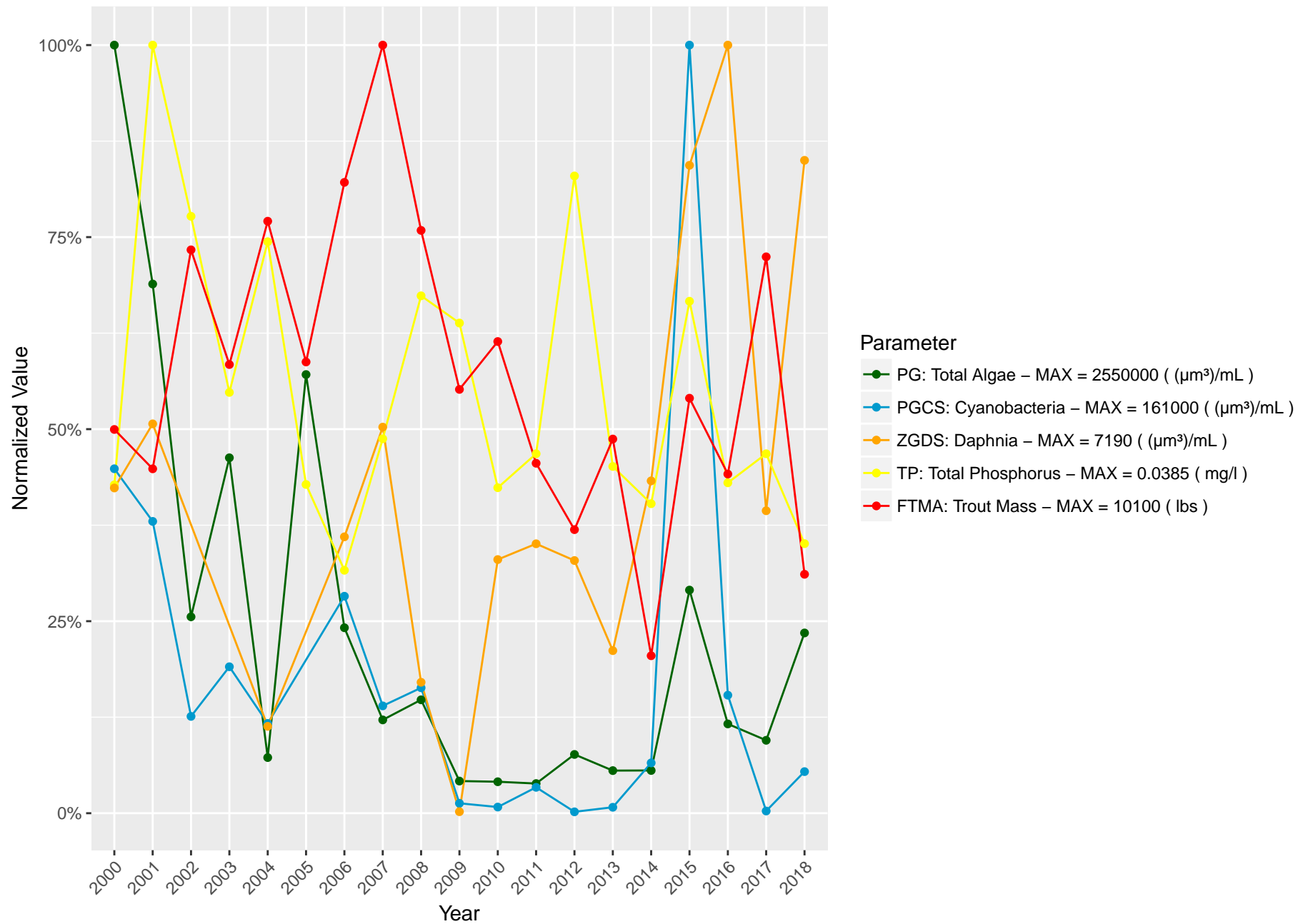
Liberty Lake – NWBOT



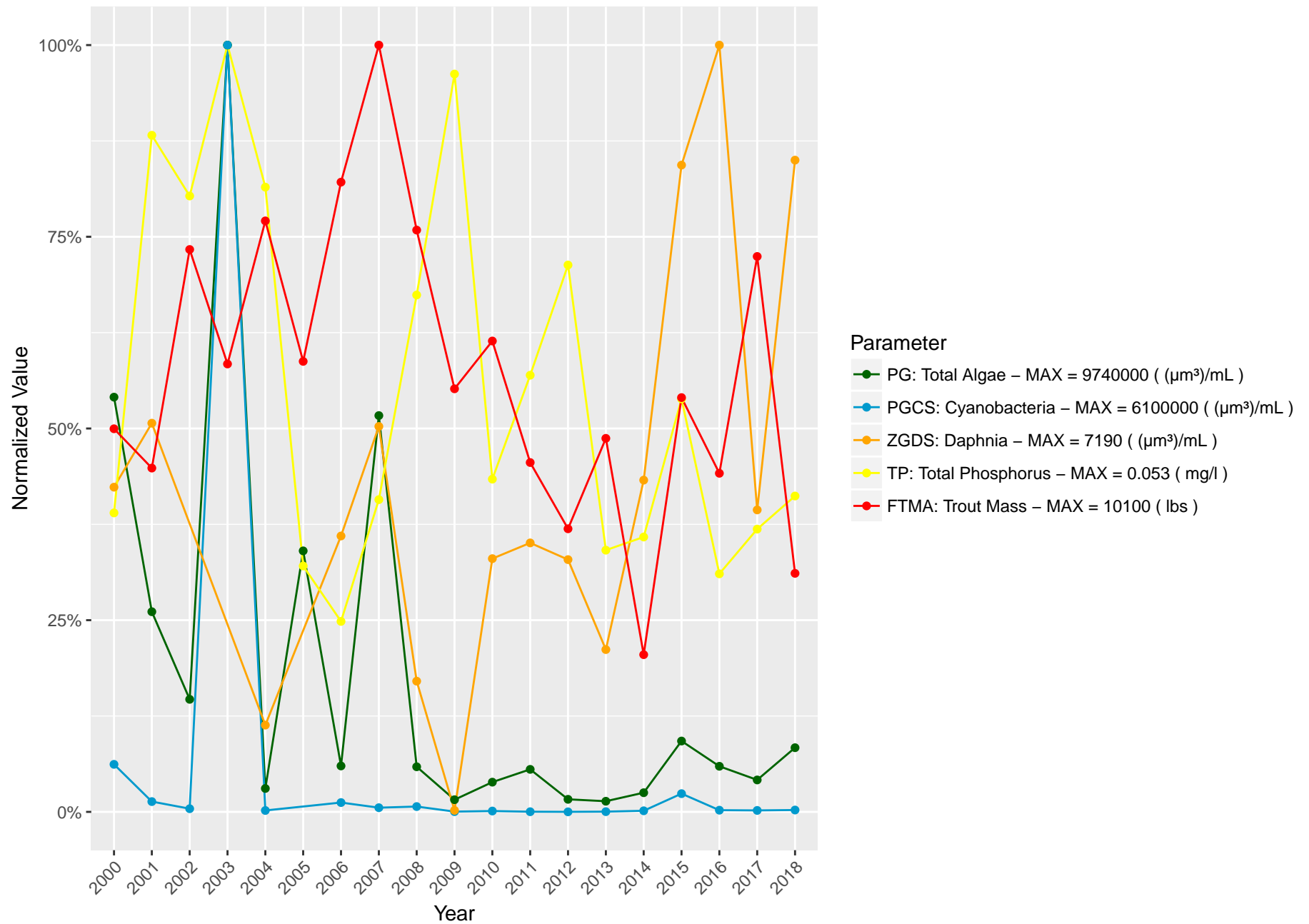
Liberty Lake – SETOP



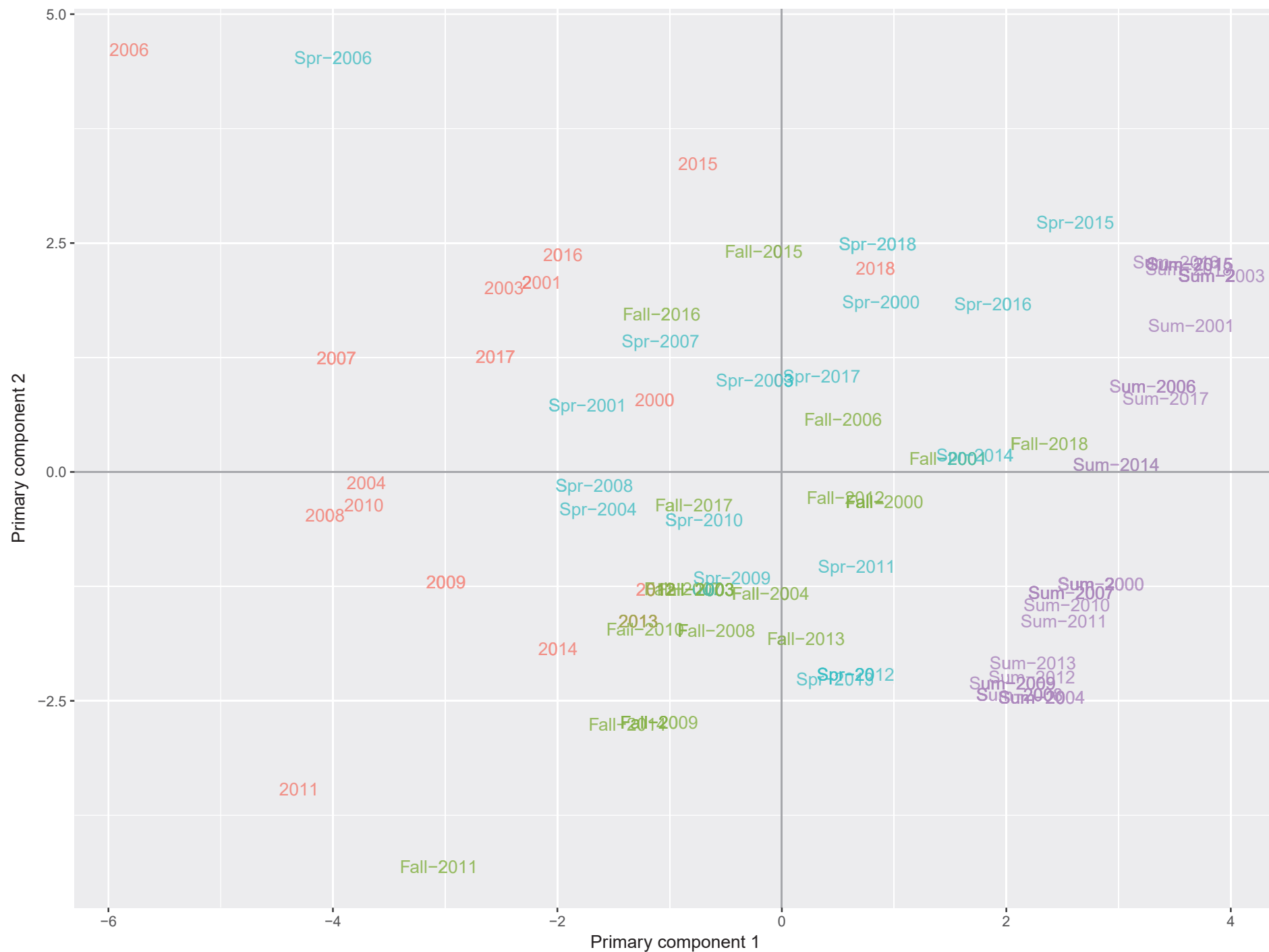
Liberty Lake – SEMID



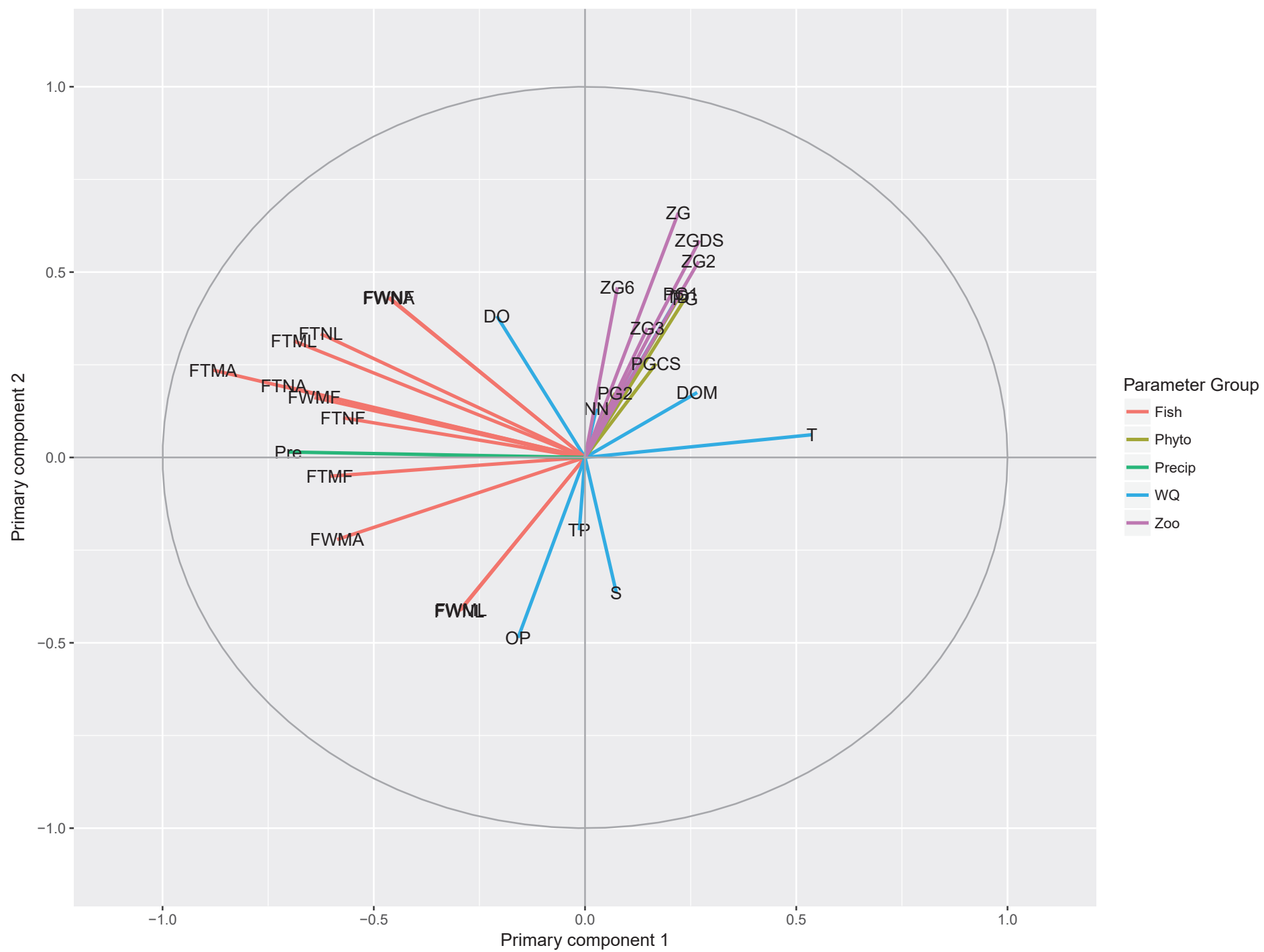
Liberty Lake – SEBOT



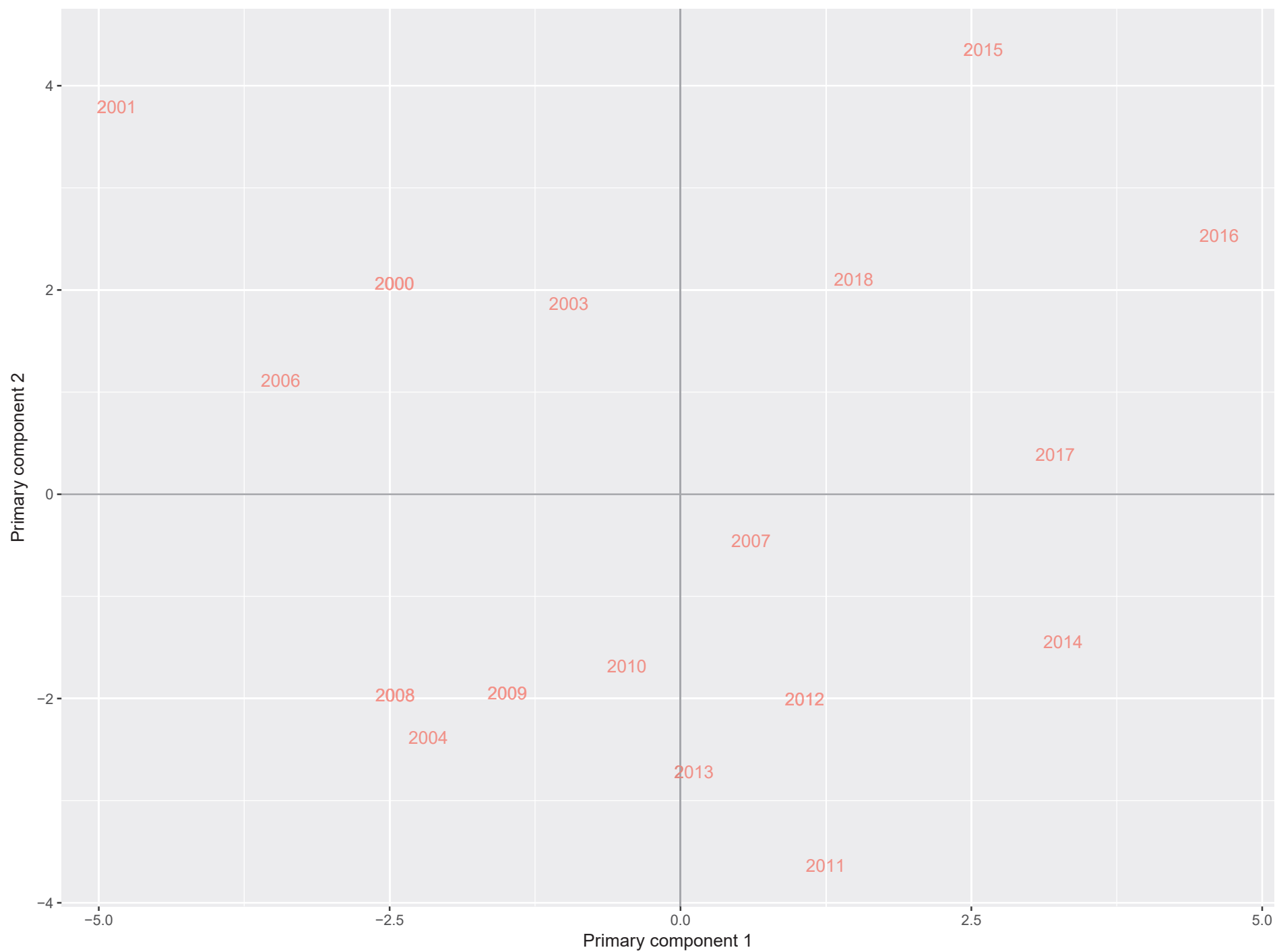
PCA plot of top depth years: All Data



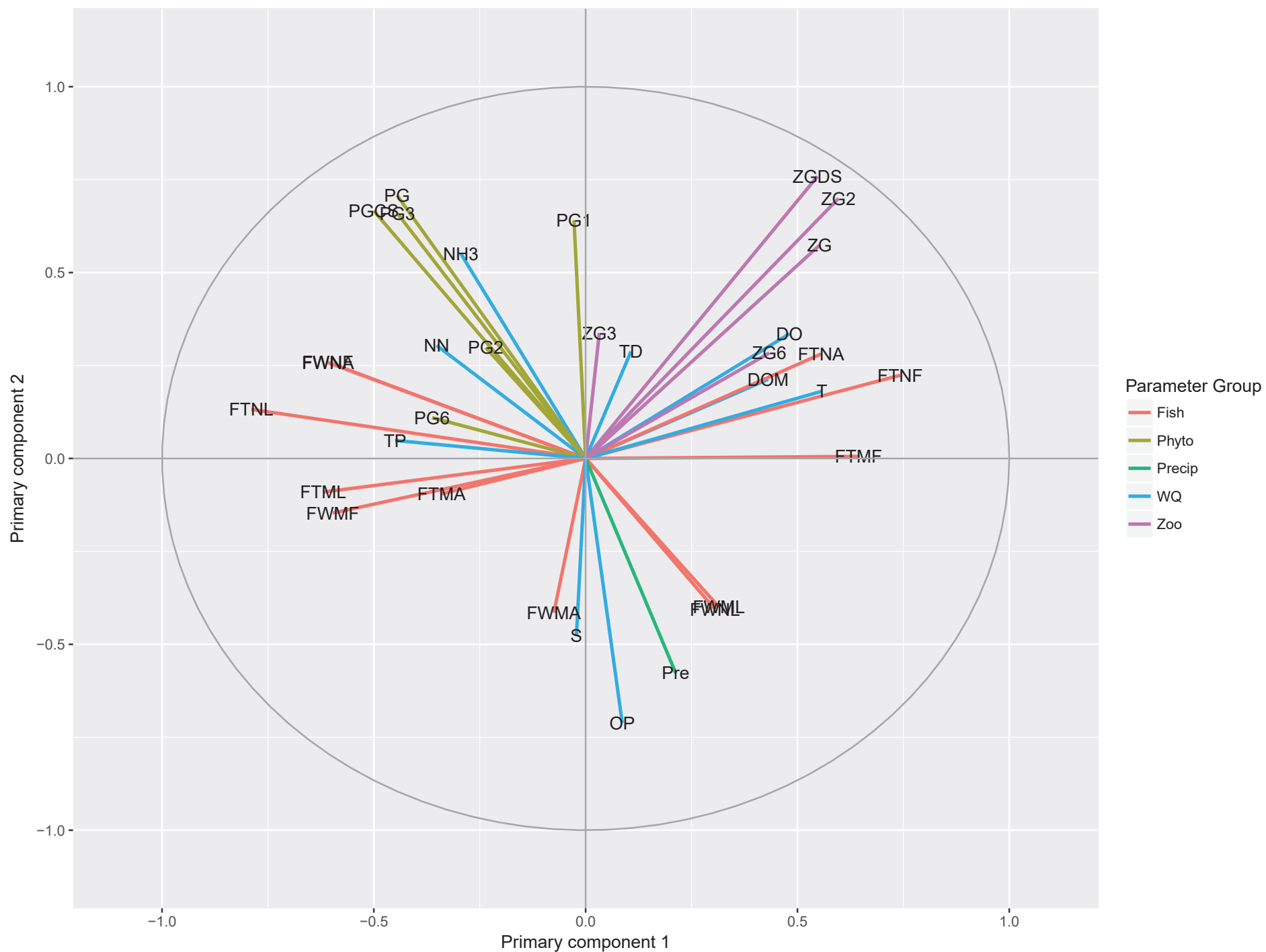
Circle of correlations: All Data



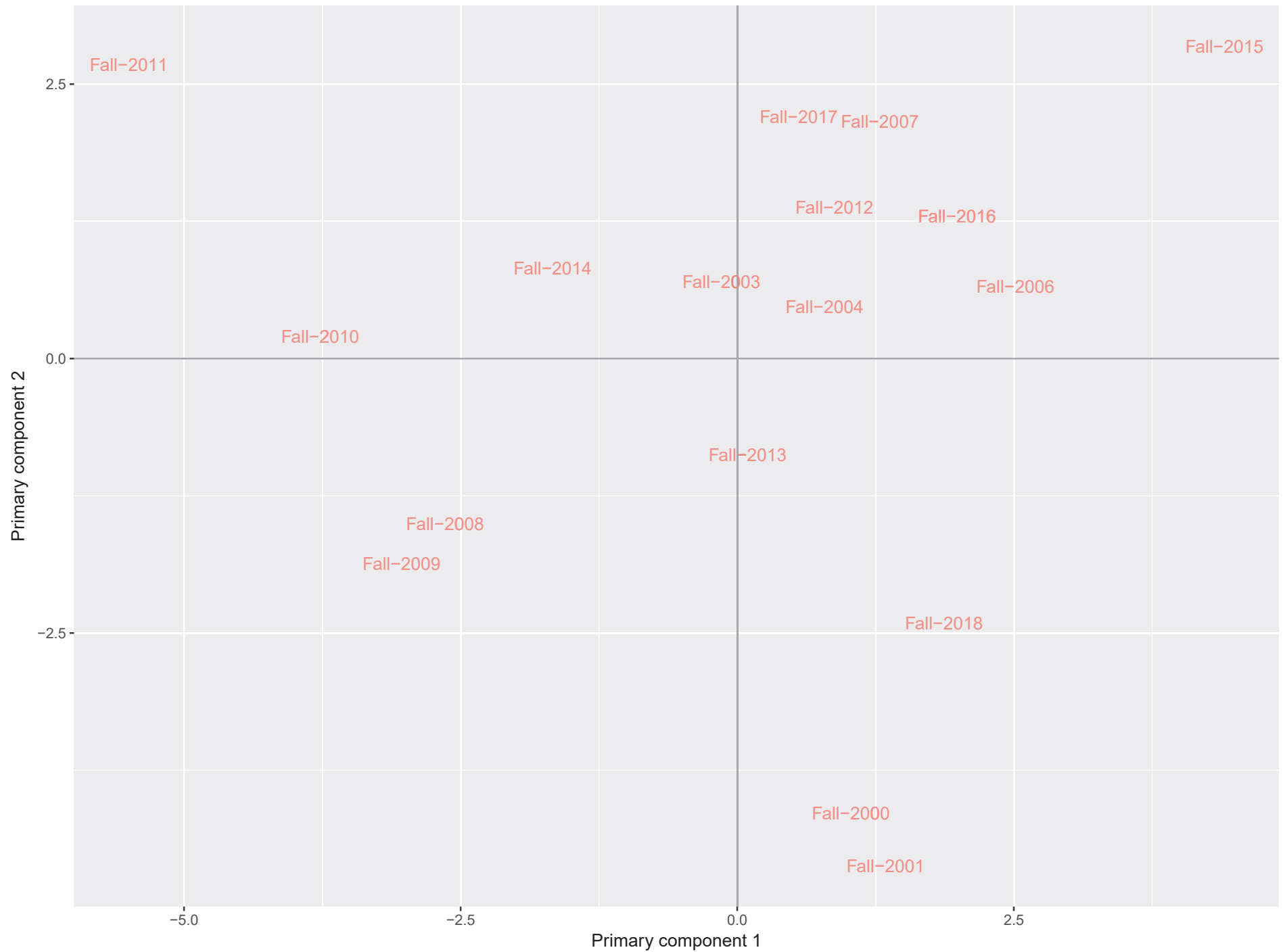
PCA plot of top depth years: Full Years



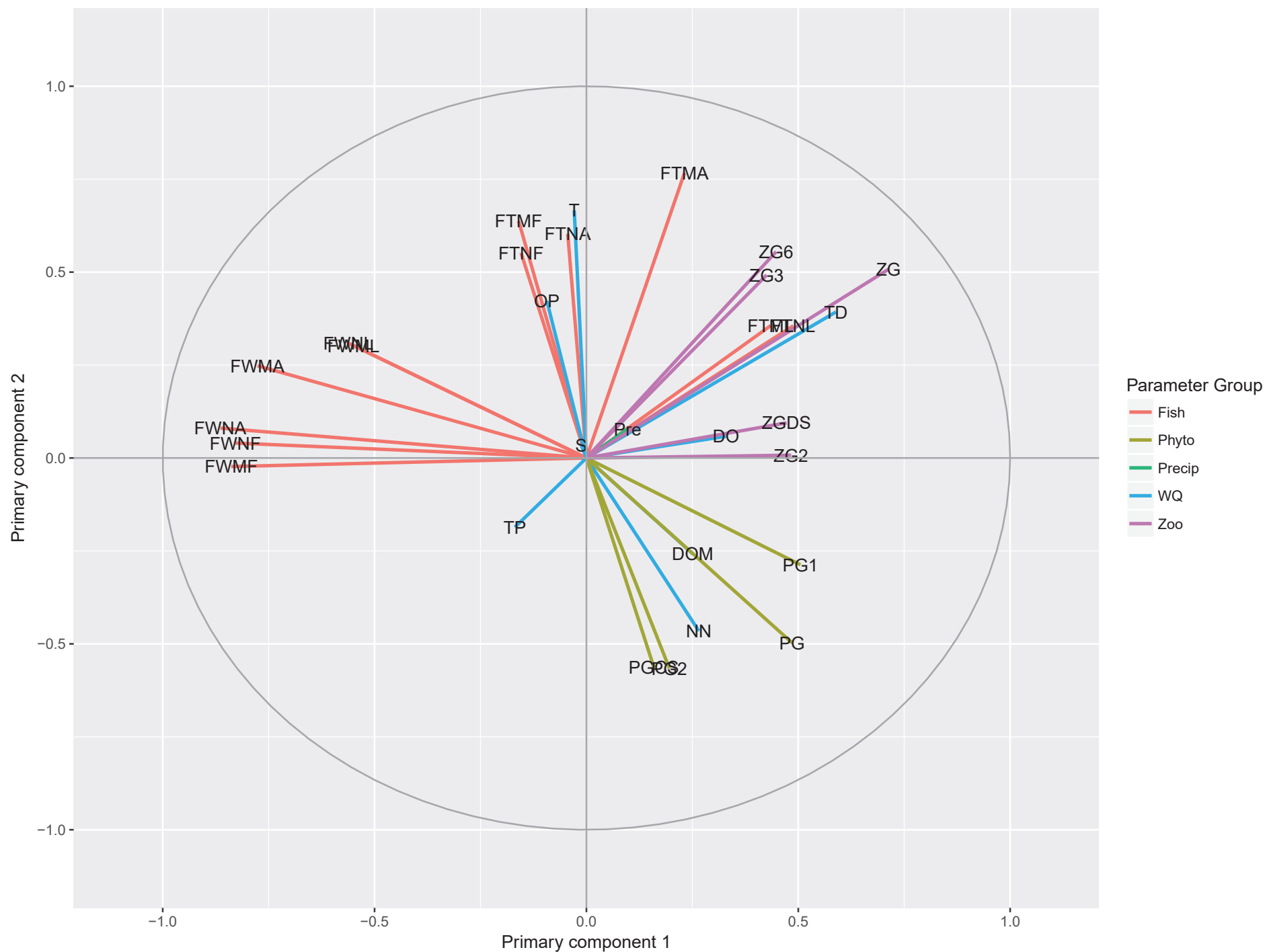
Circle of correlations: Full Years



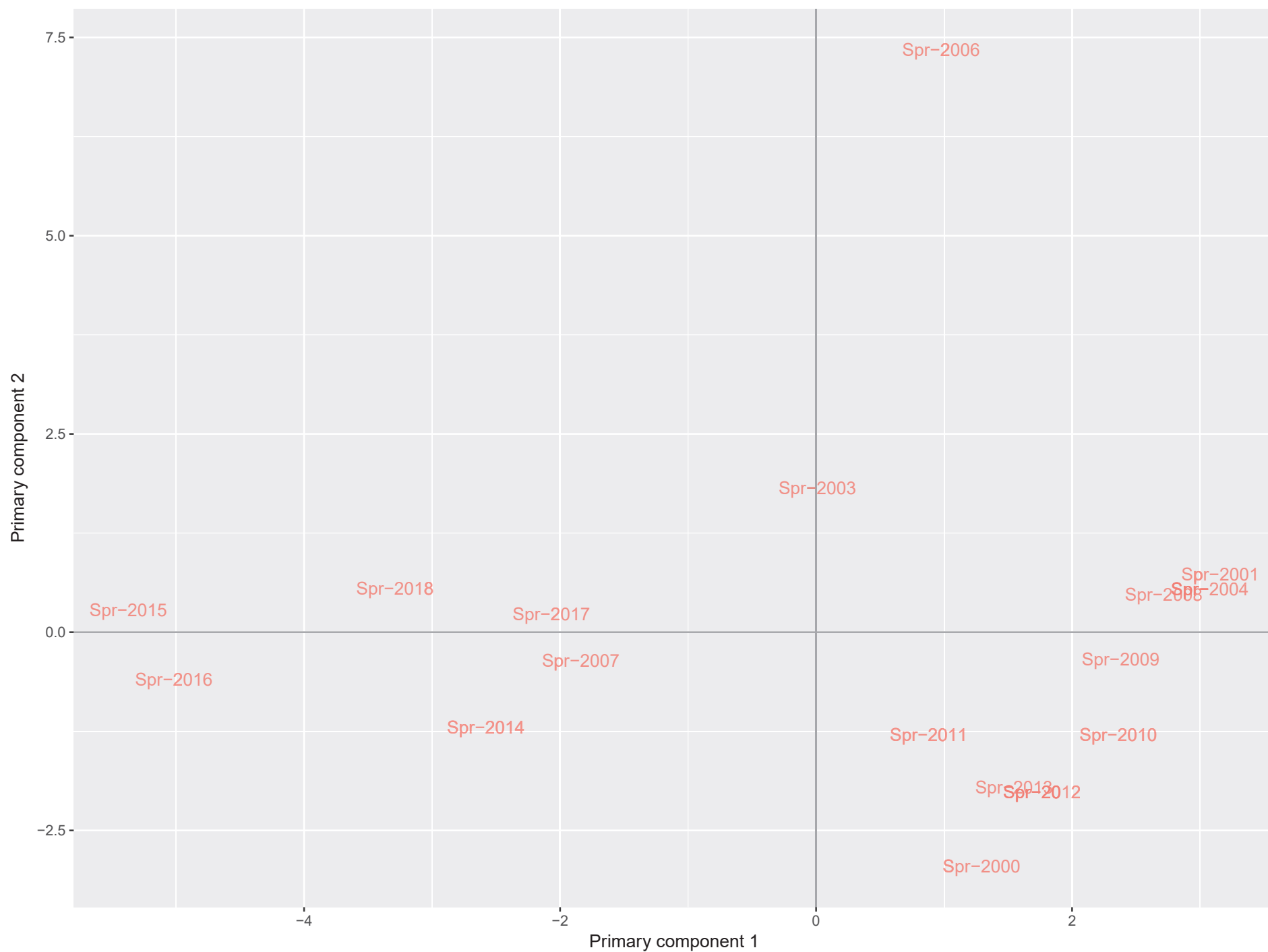
PCA plot of top depth years: Fall



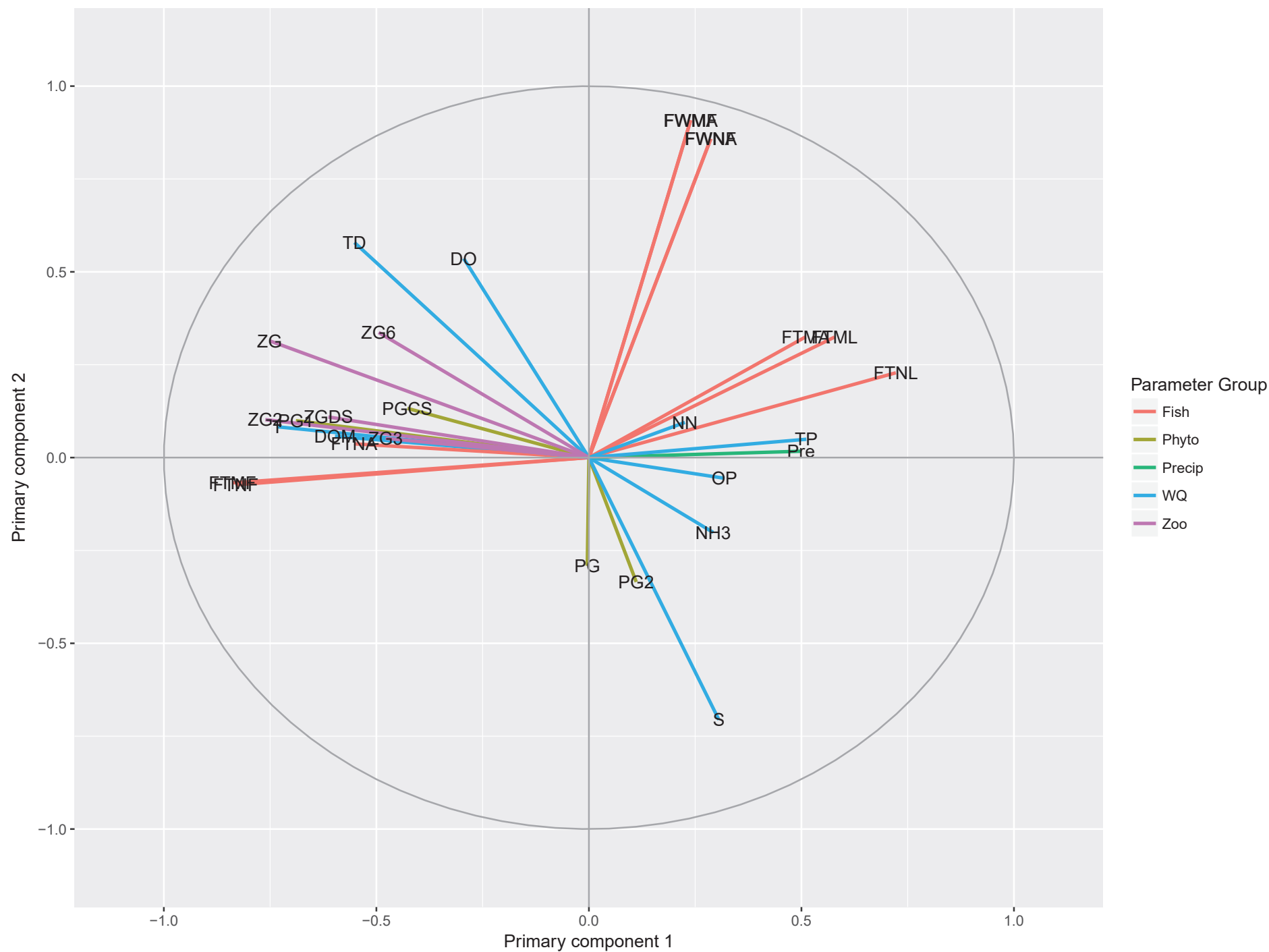
Circle of correlations: Fall



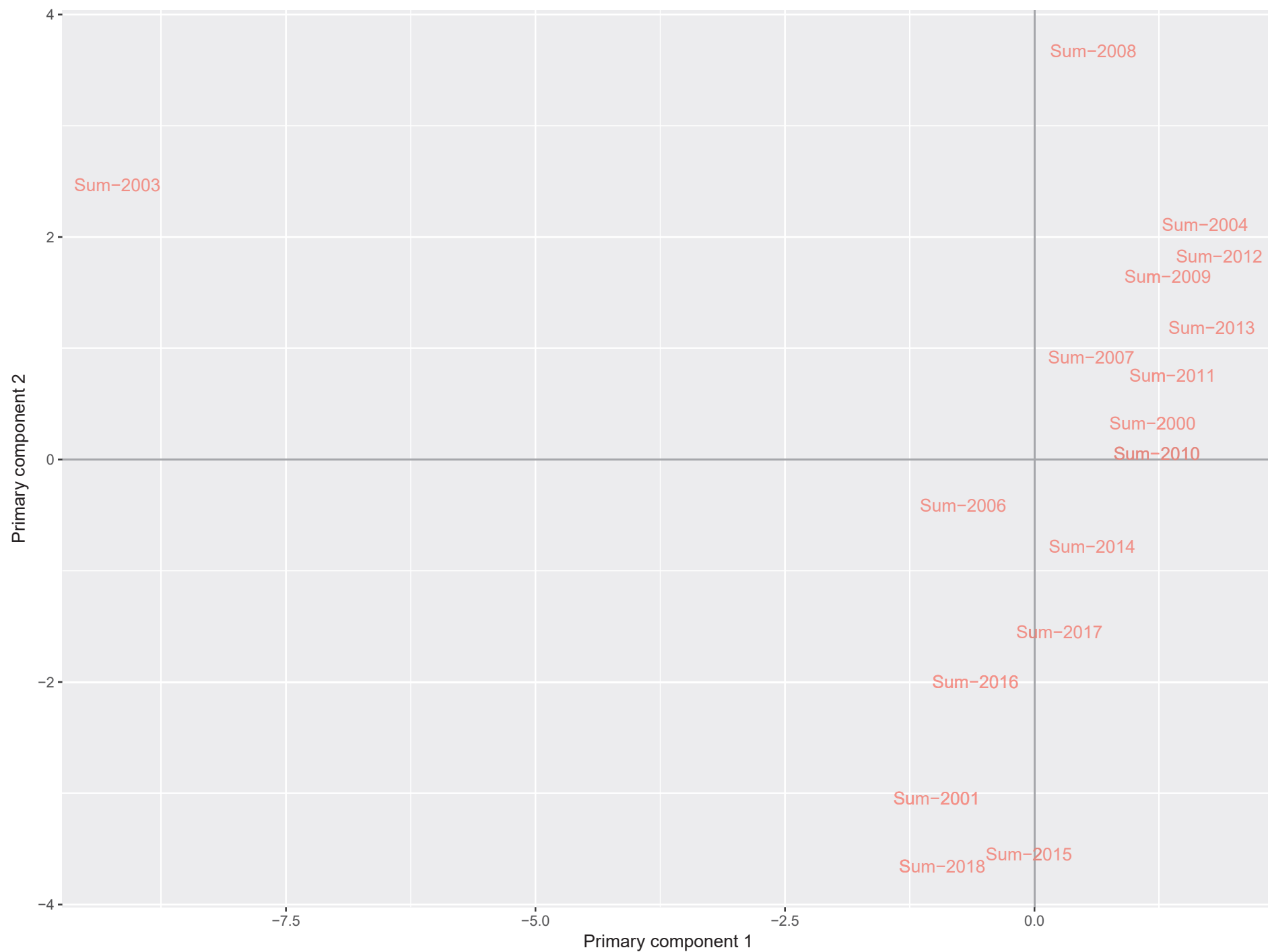
PCA plot of top depth years: Spring



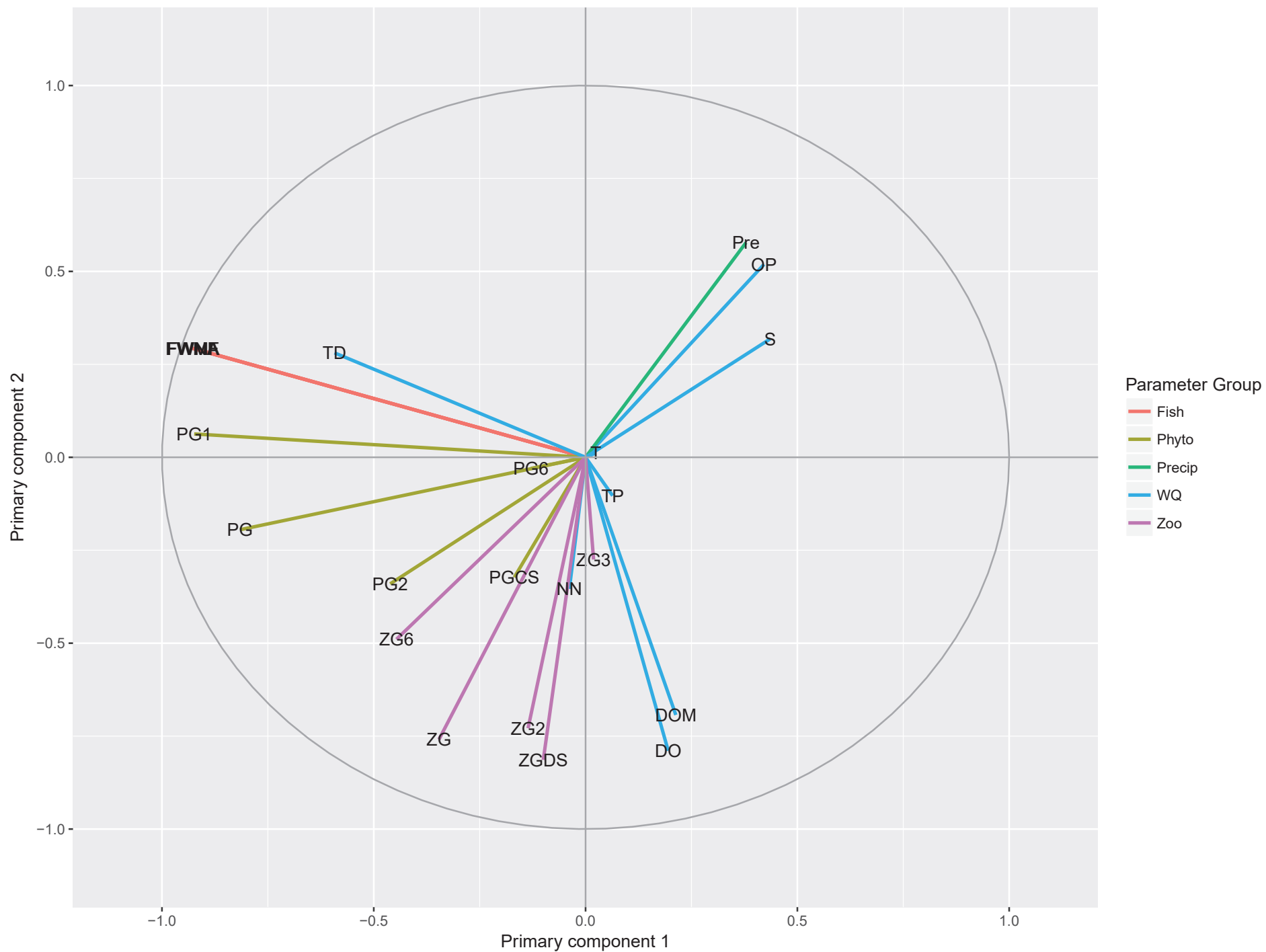
Circle of correlations: Spring



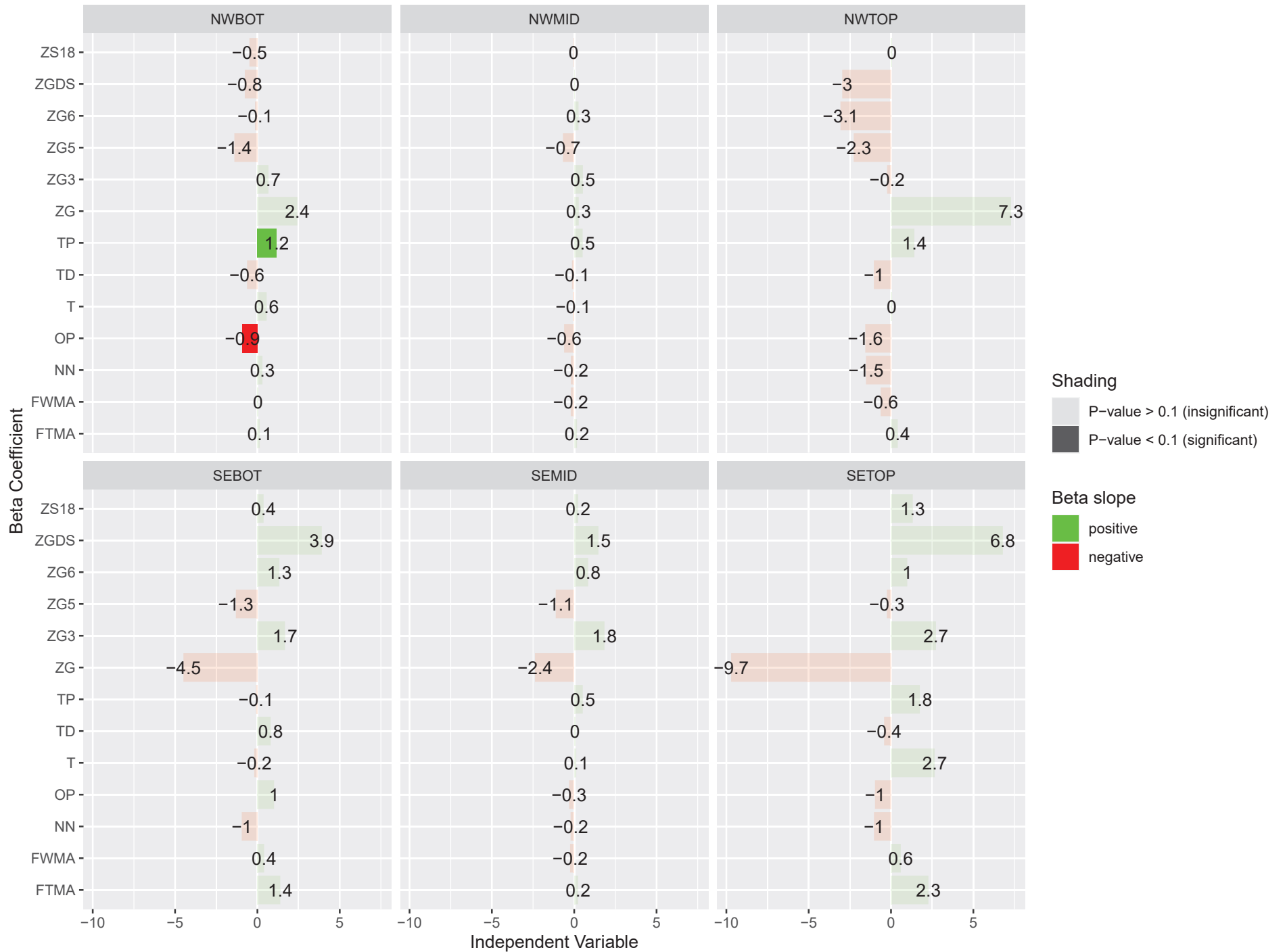
PCA plot of top depth years: Summer



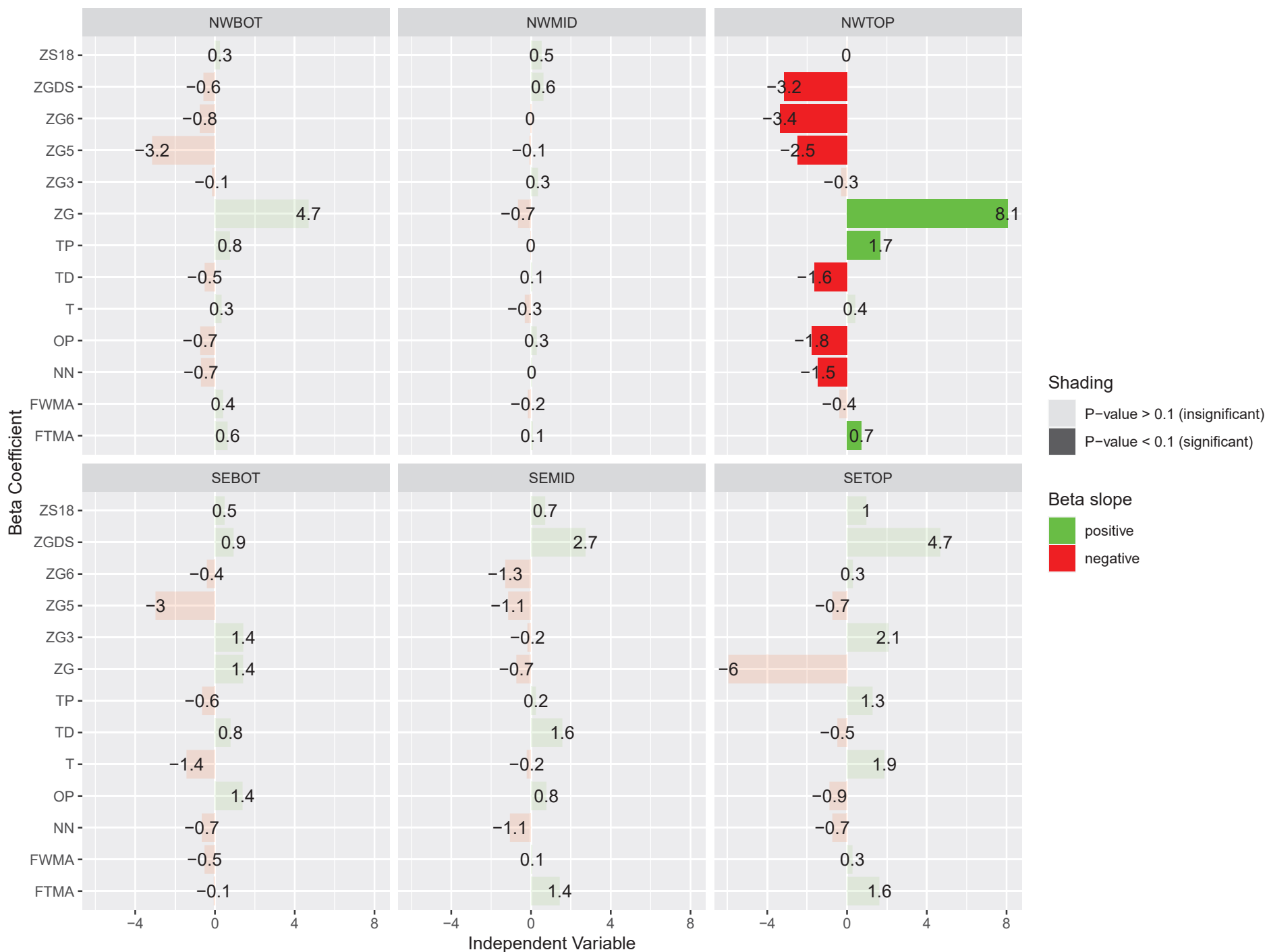
Circle of correlations: Summer



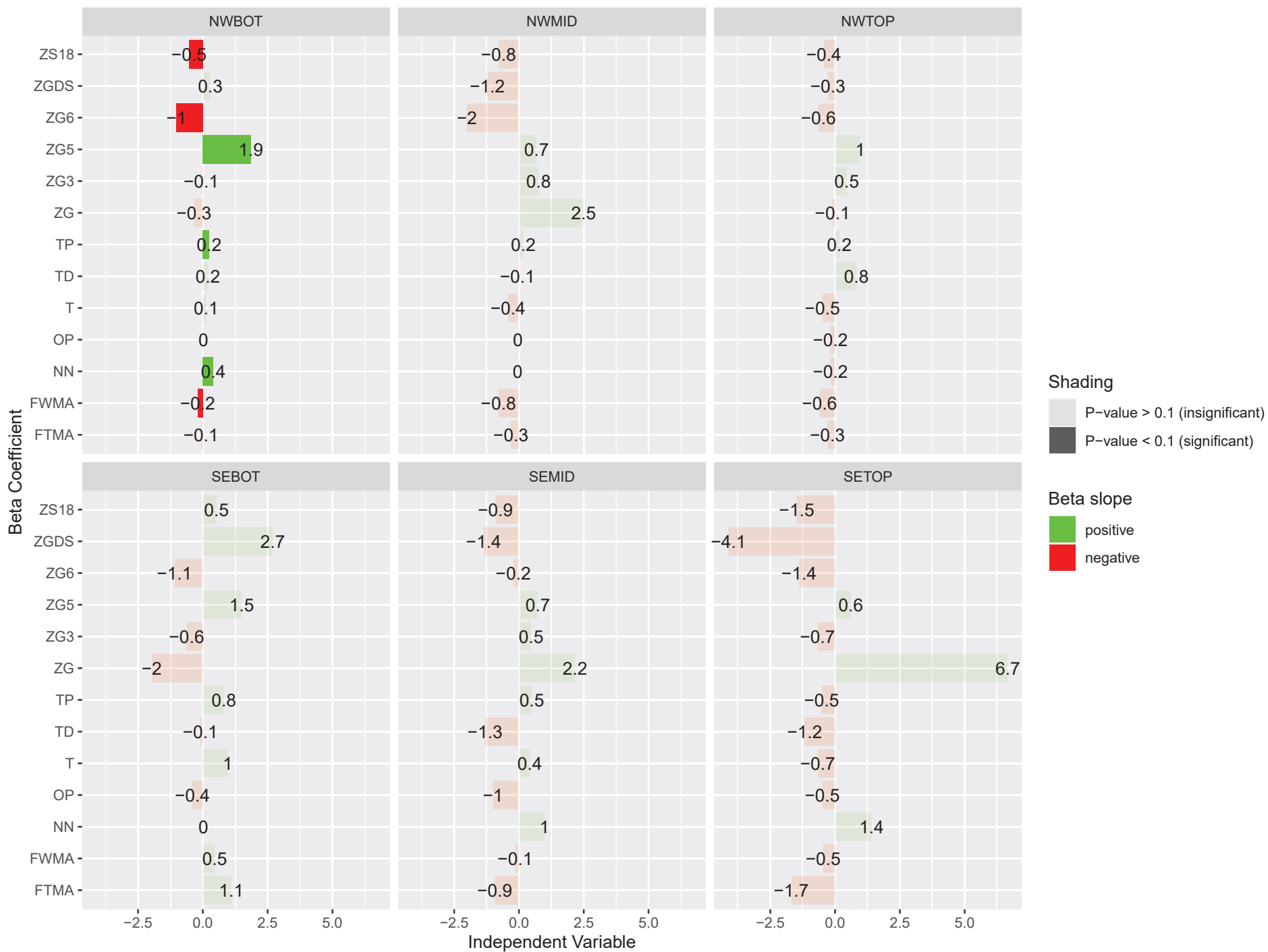
Multiple Regression Results for PG



Multiple Regression Results for PGCS



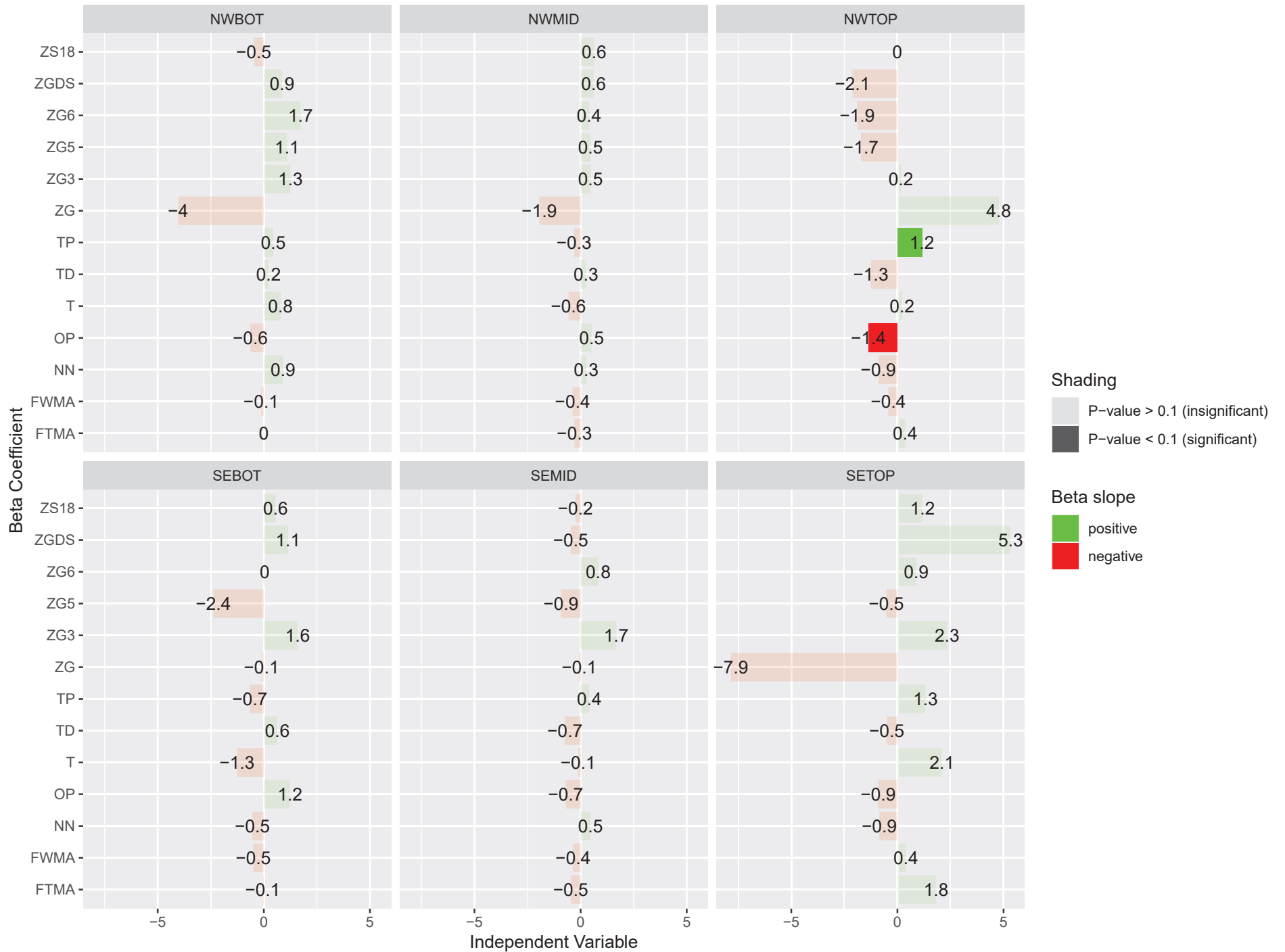
Multiple Regression Results for PS1



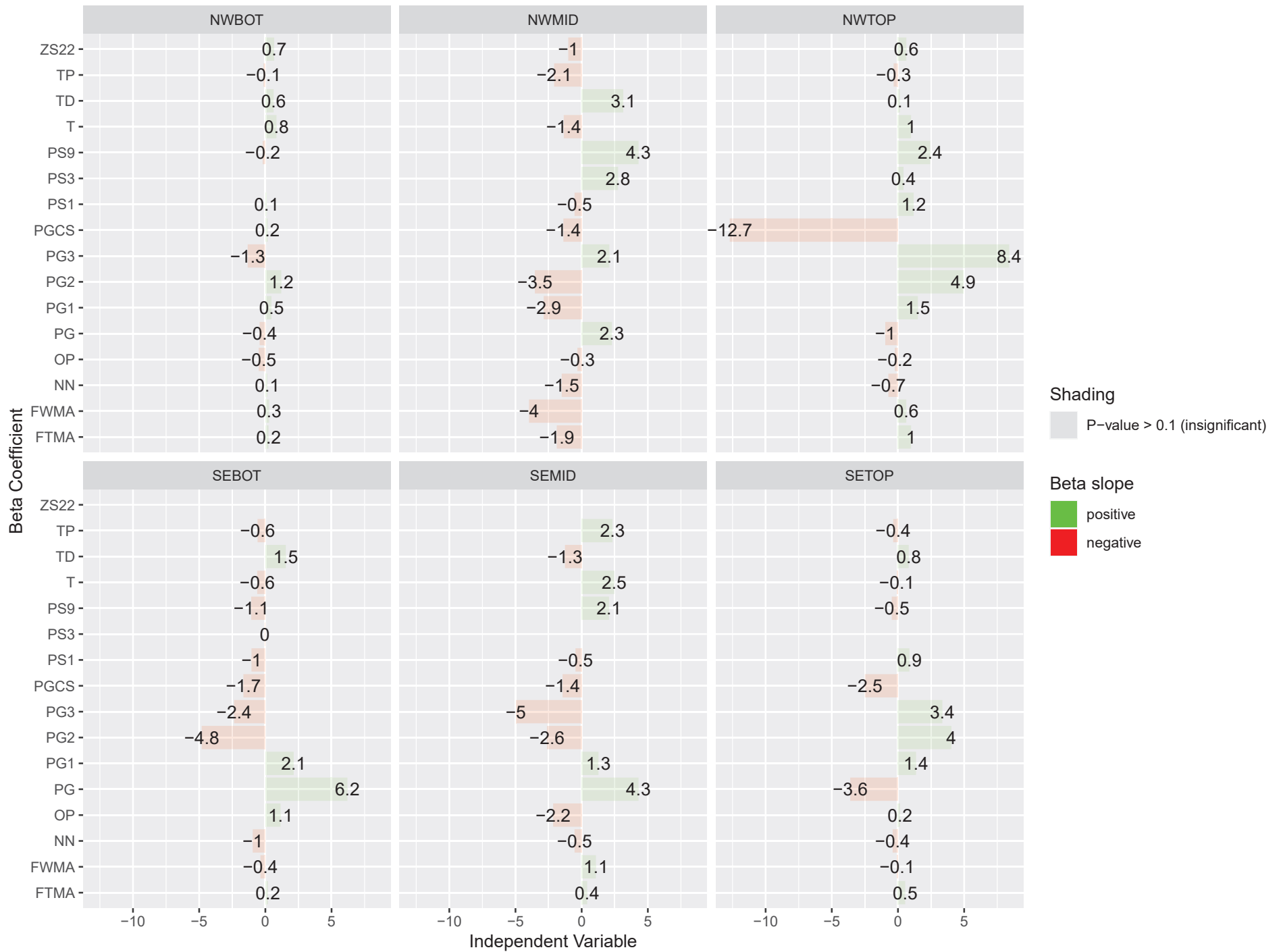
Multiple Regression Results for PS3



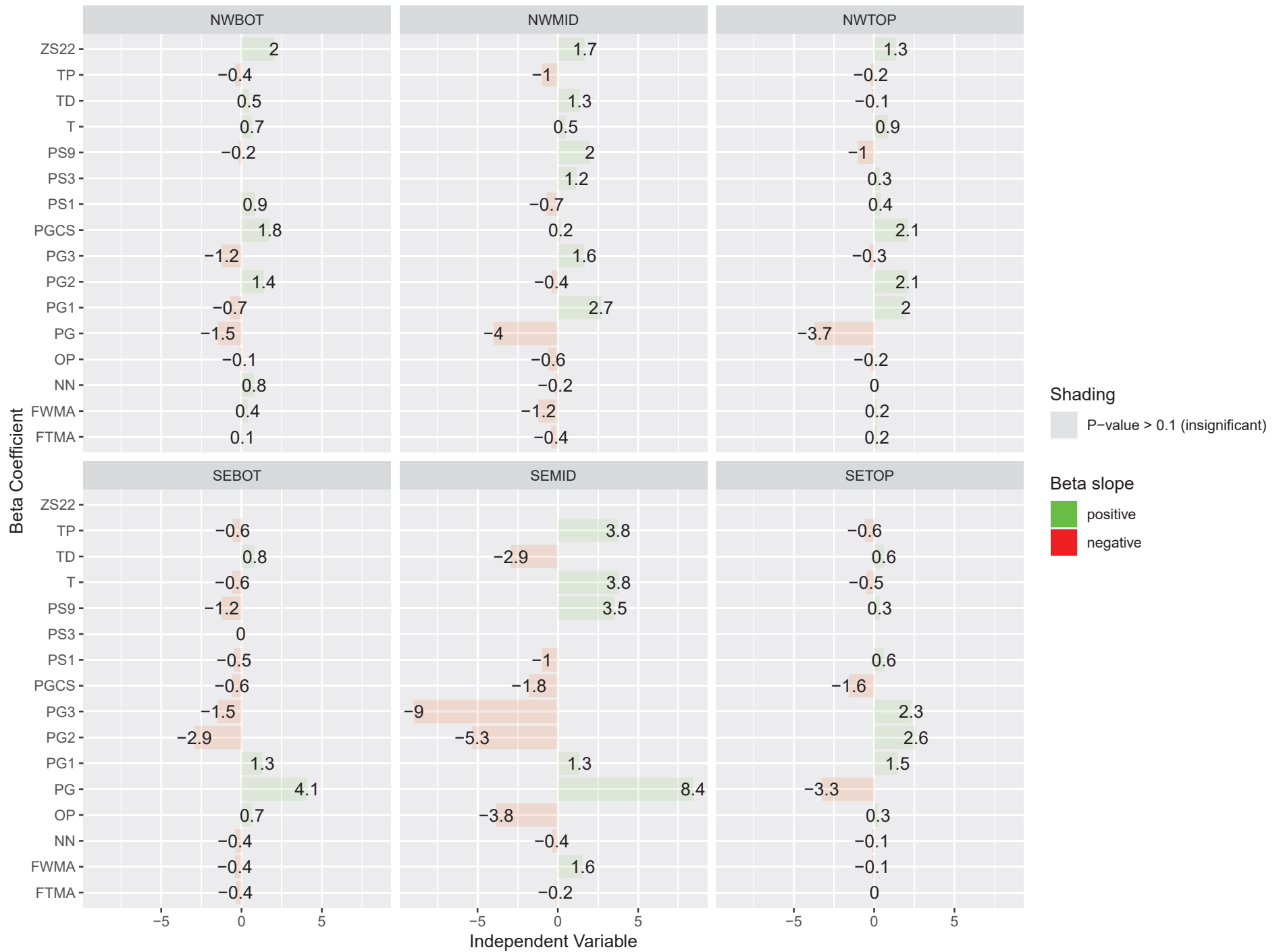
Multiple Regression Results for PS9



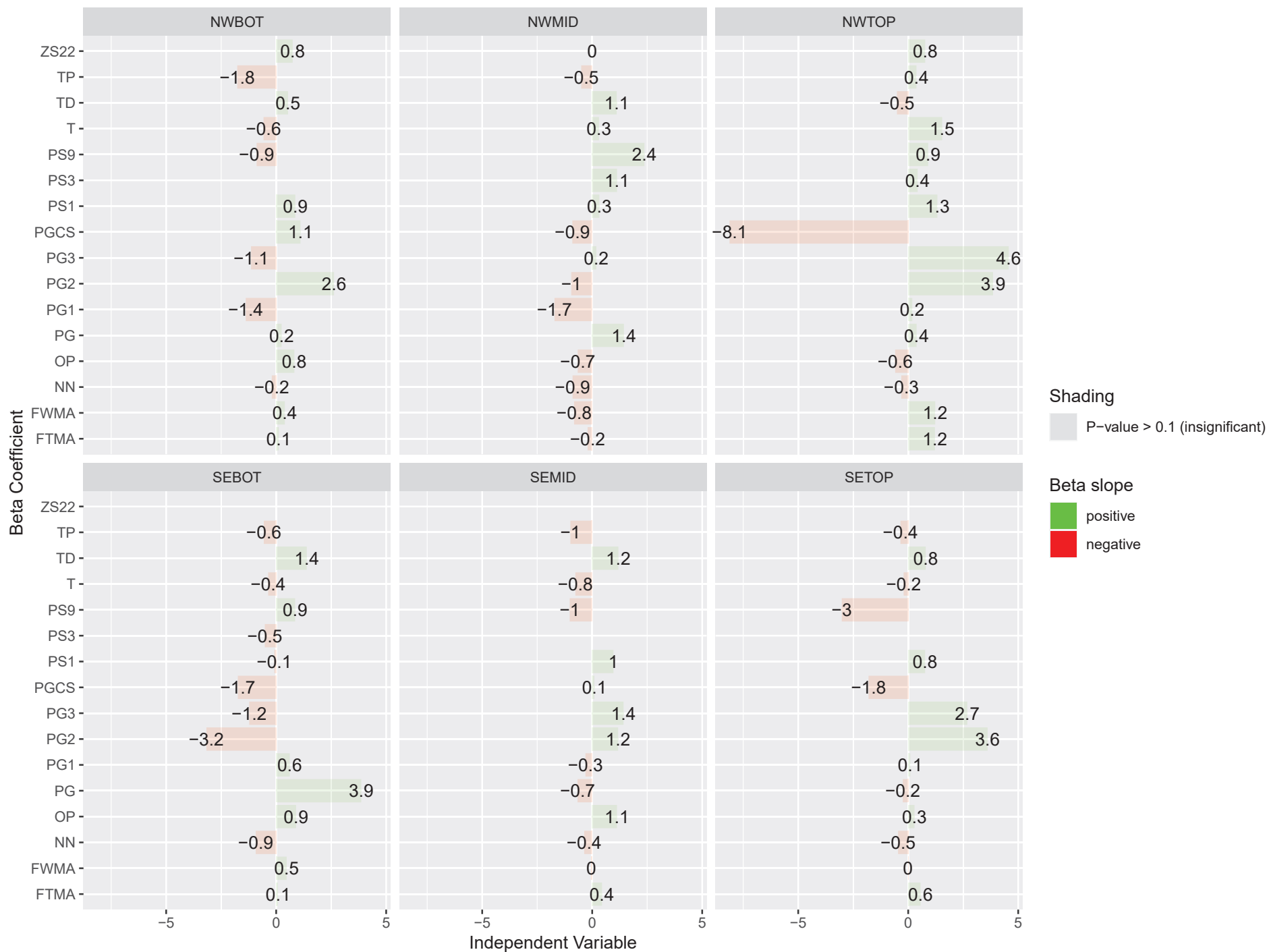
Multiple Regression Results for ZG



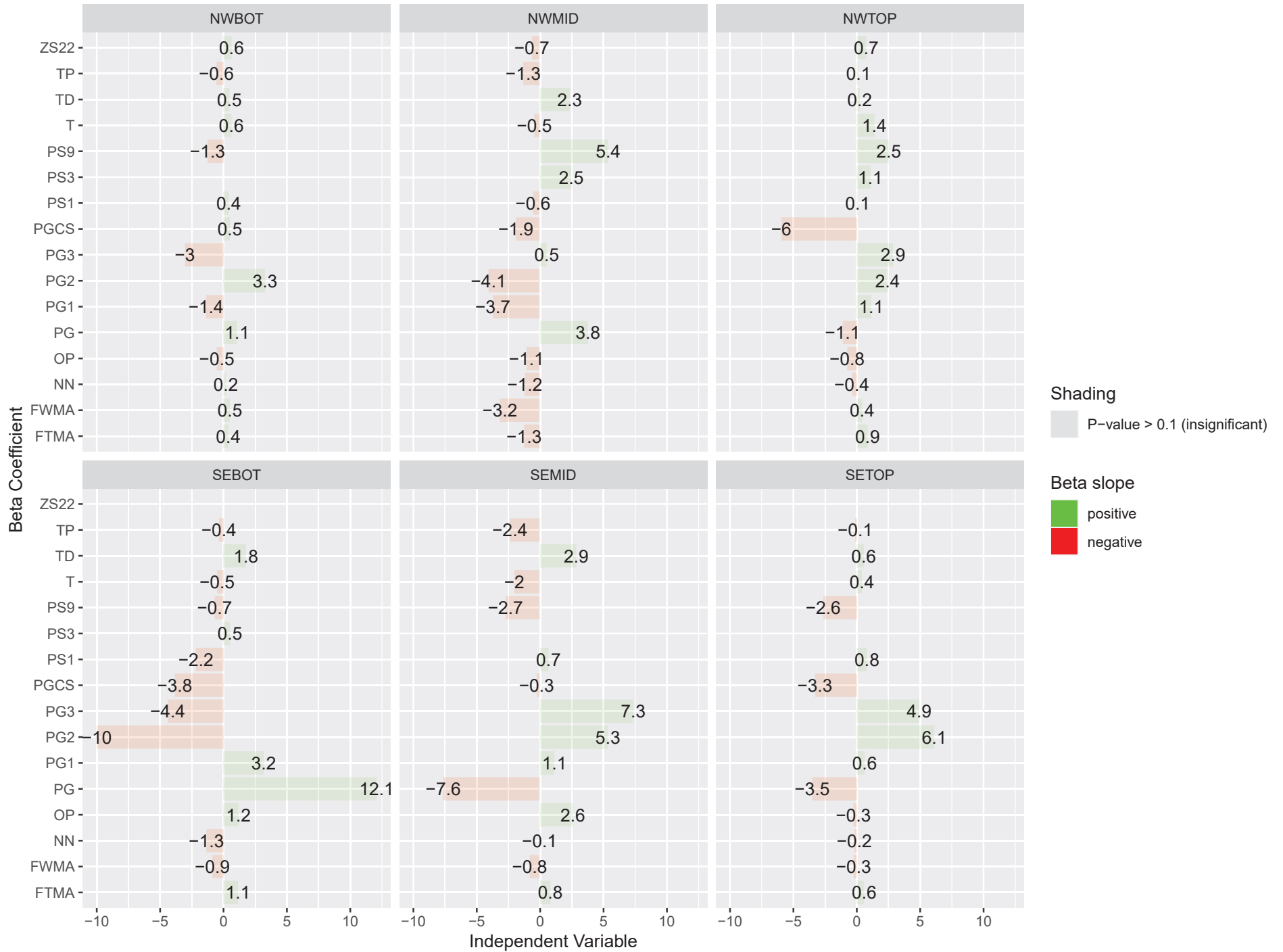
Multiple Regression Results for ZGDS



Multiple Regression Results for ZG3

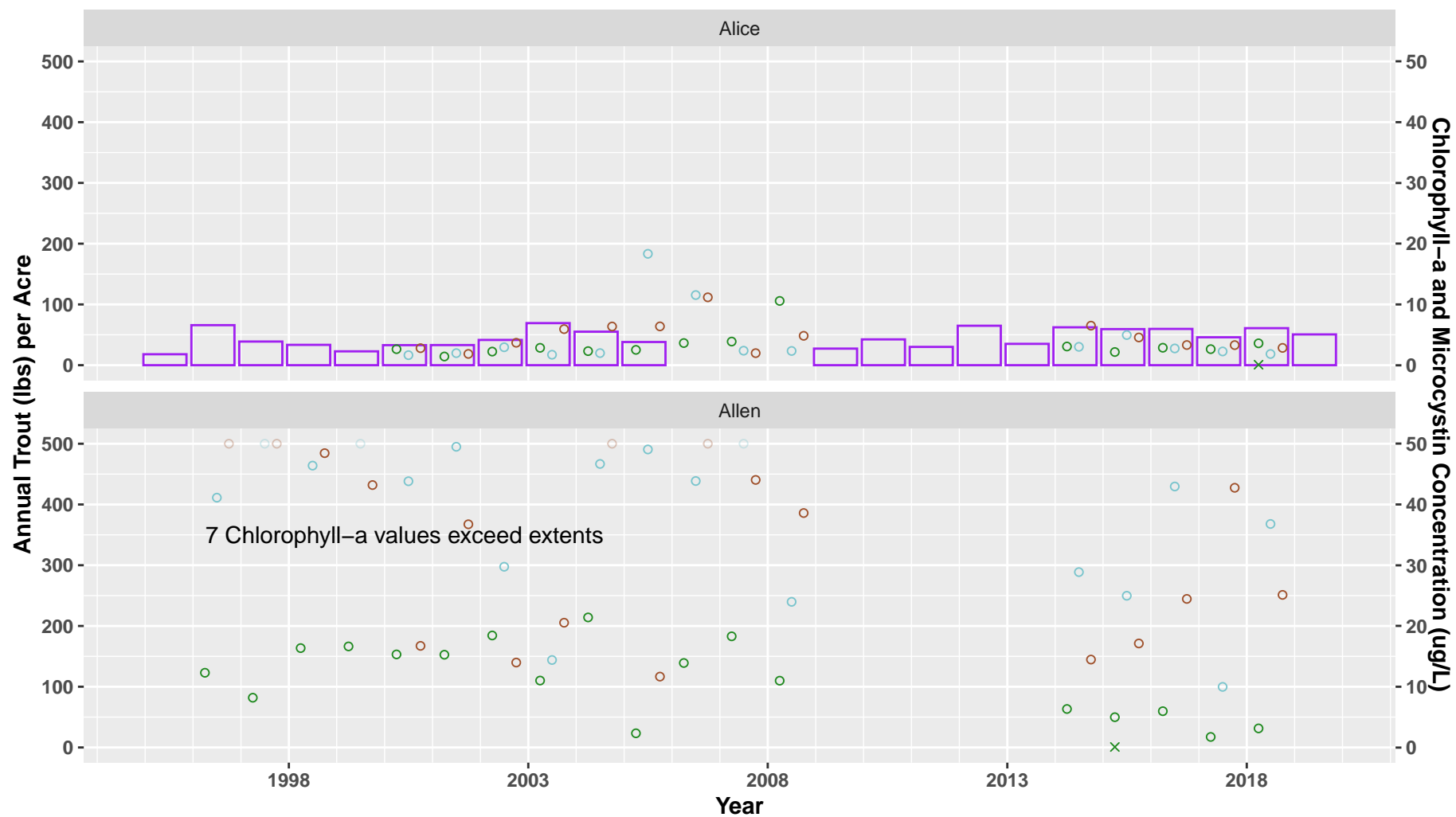


Multiple Regression Results for ZS18



APPENDIX D

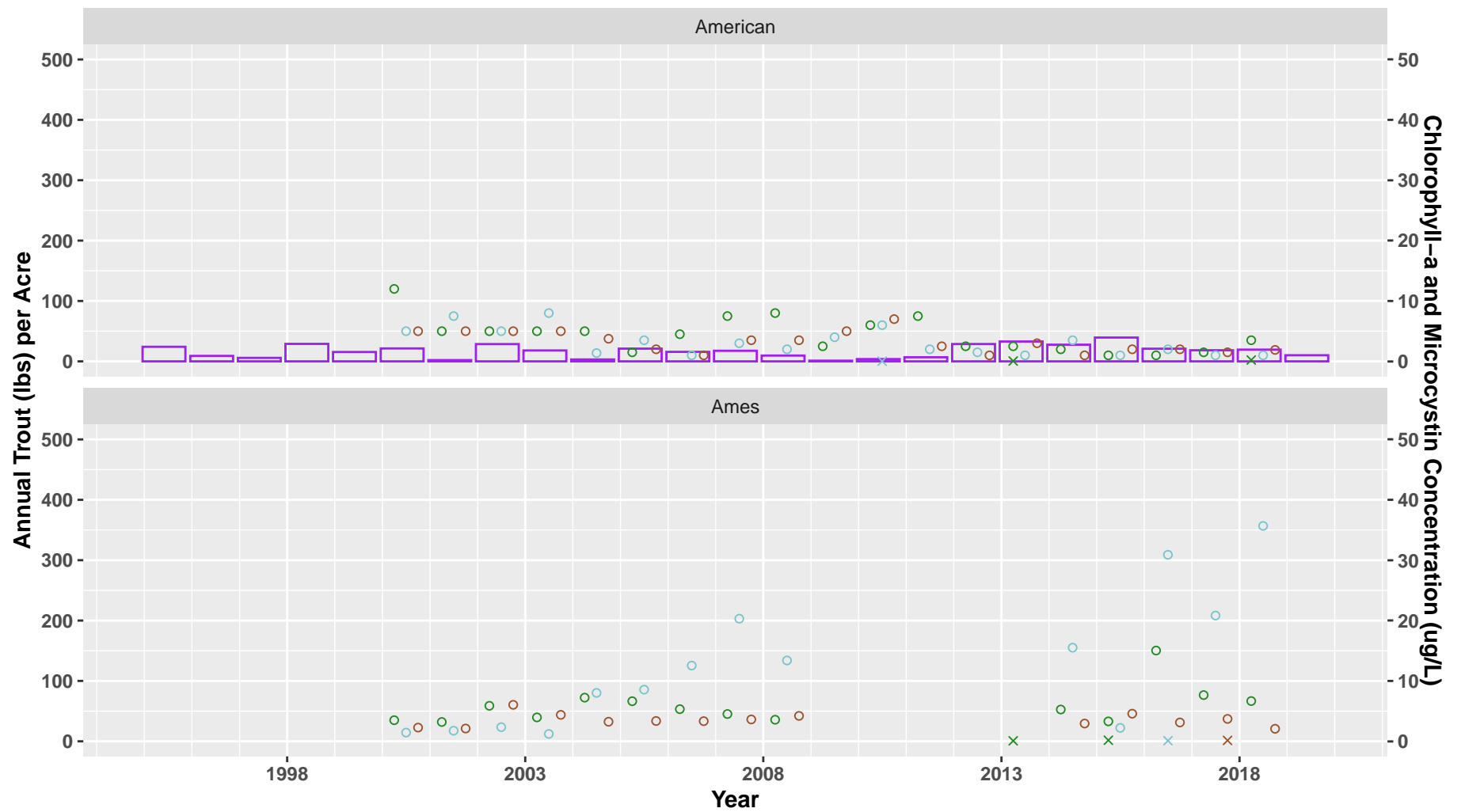
Washington Lakes Figures



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

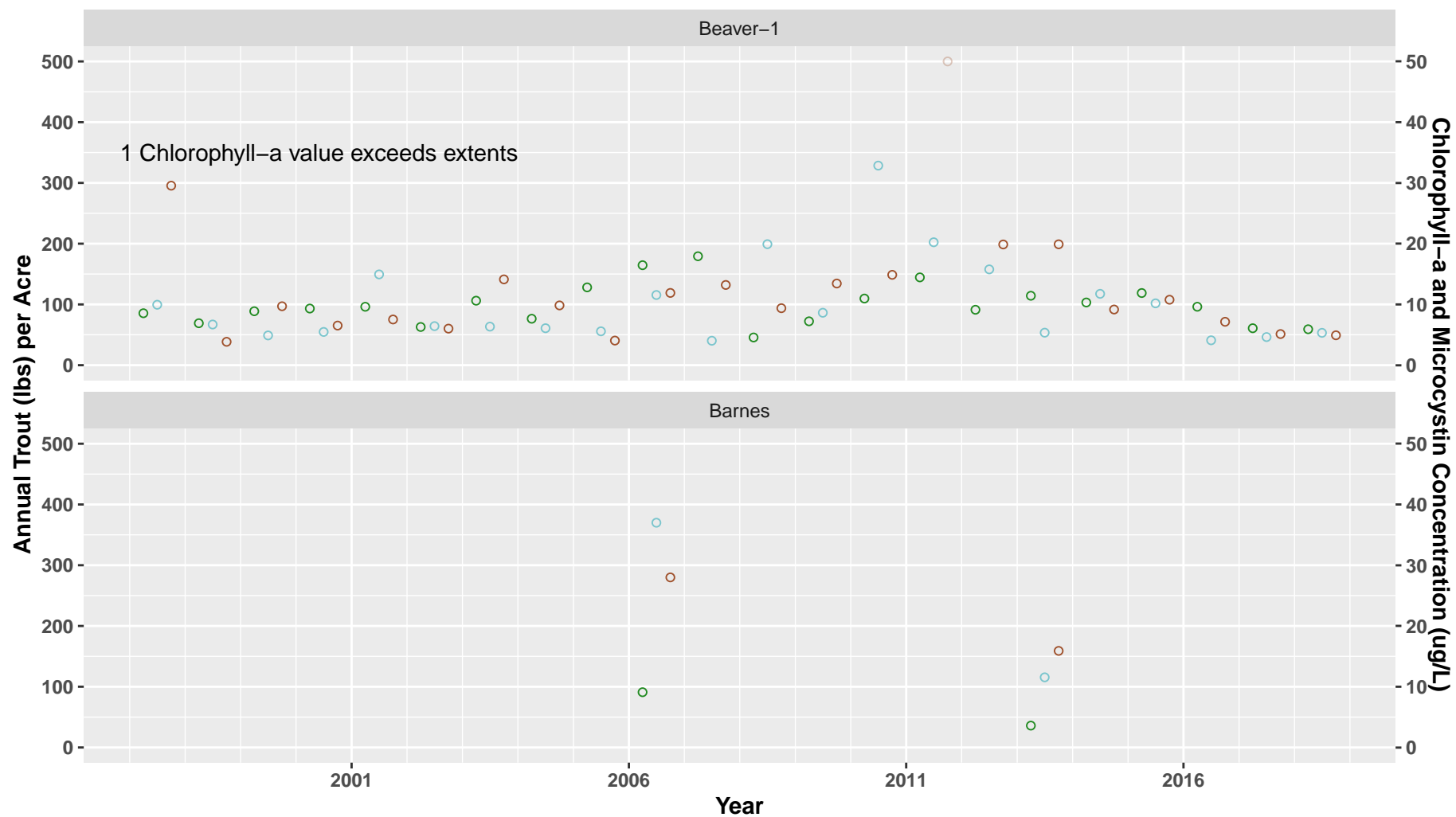
Shading ● within extents



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

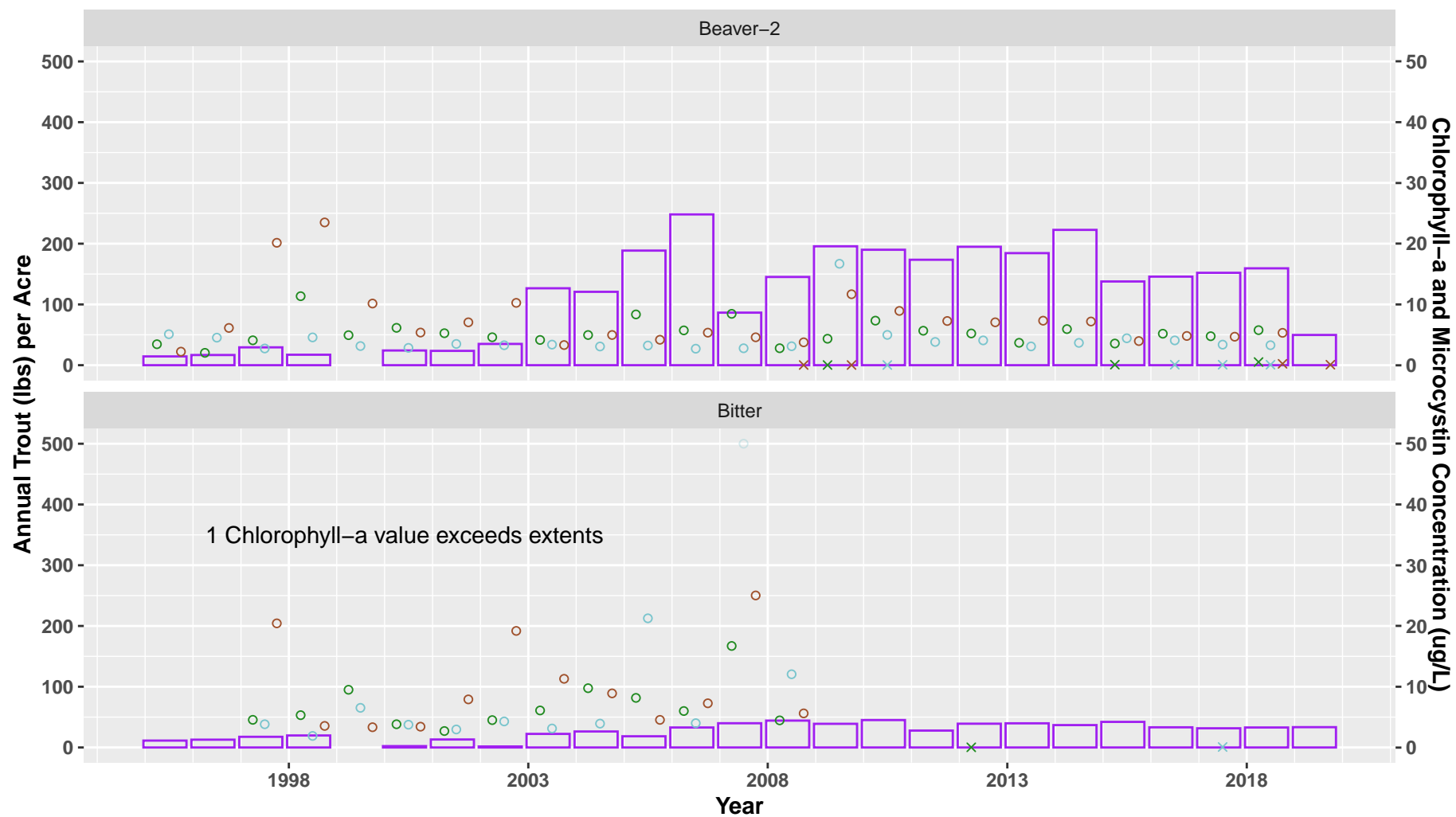
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Trout lbs per Acre

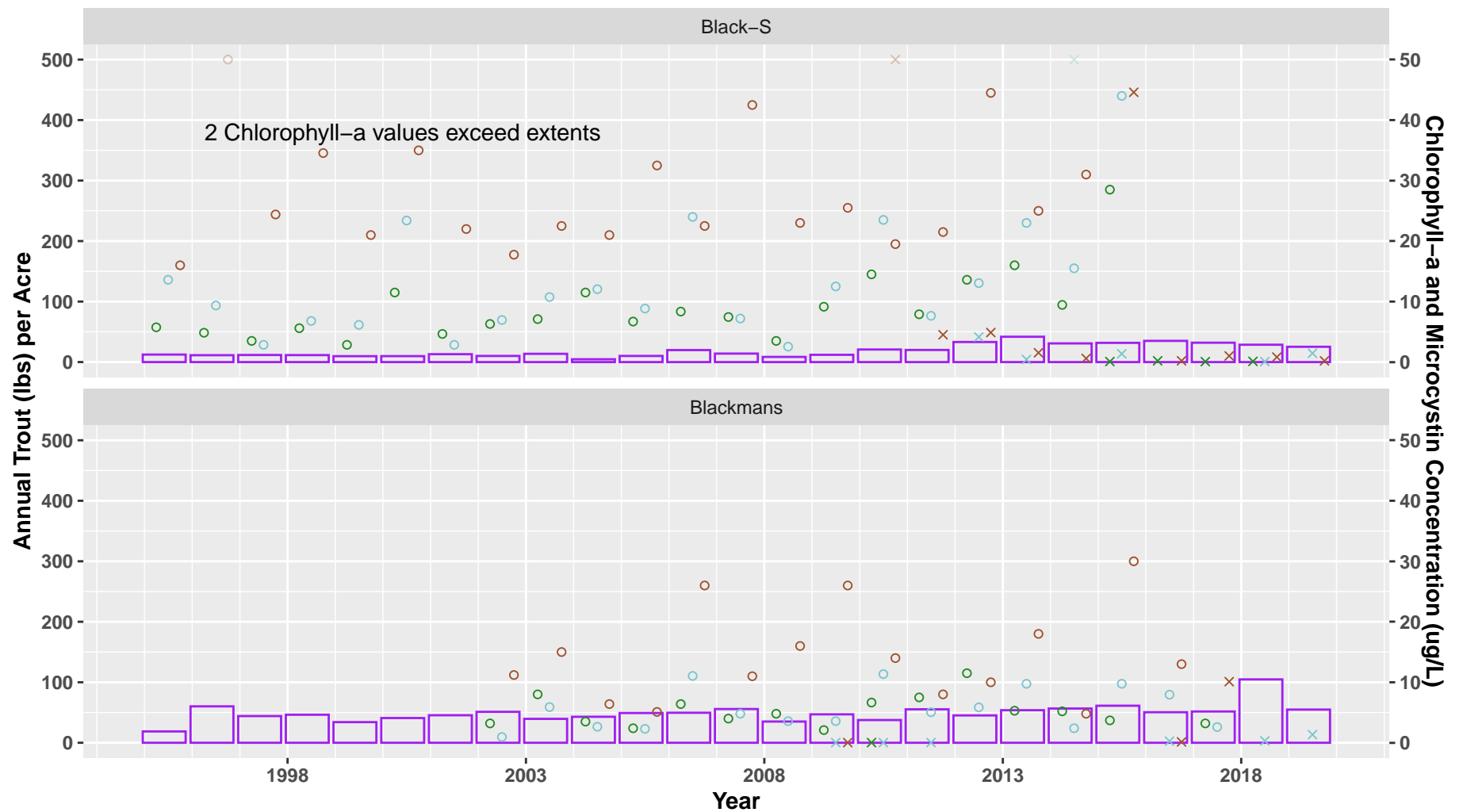
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

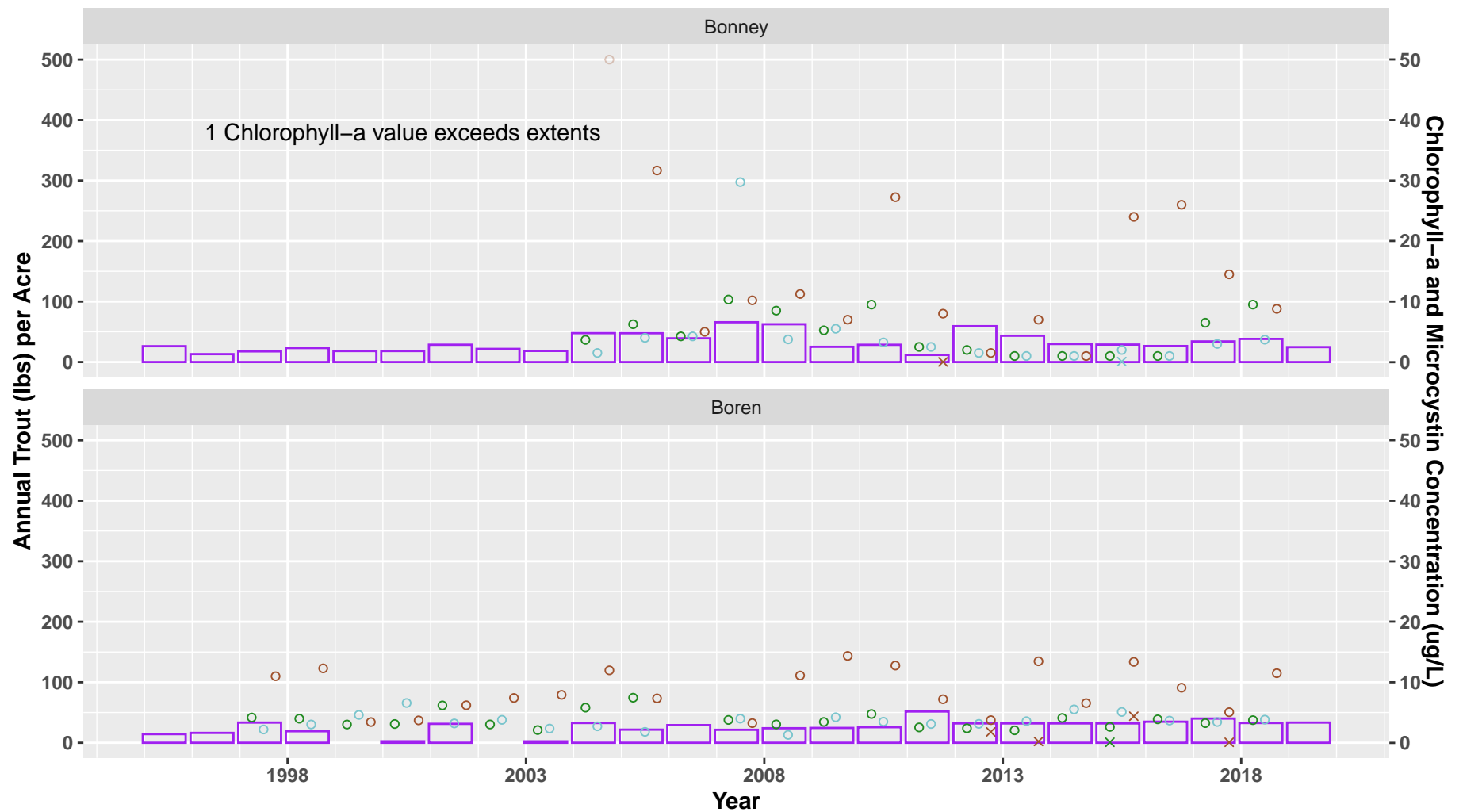
Season ● Spring ● Summer ● Fall

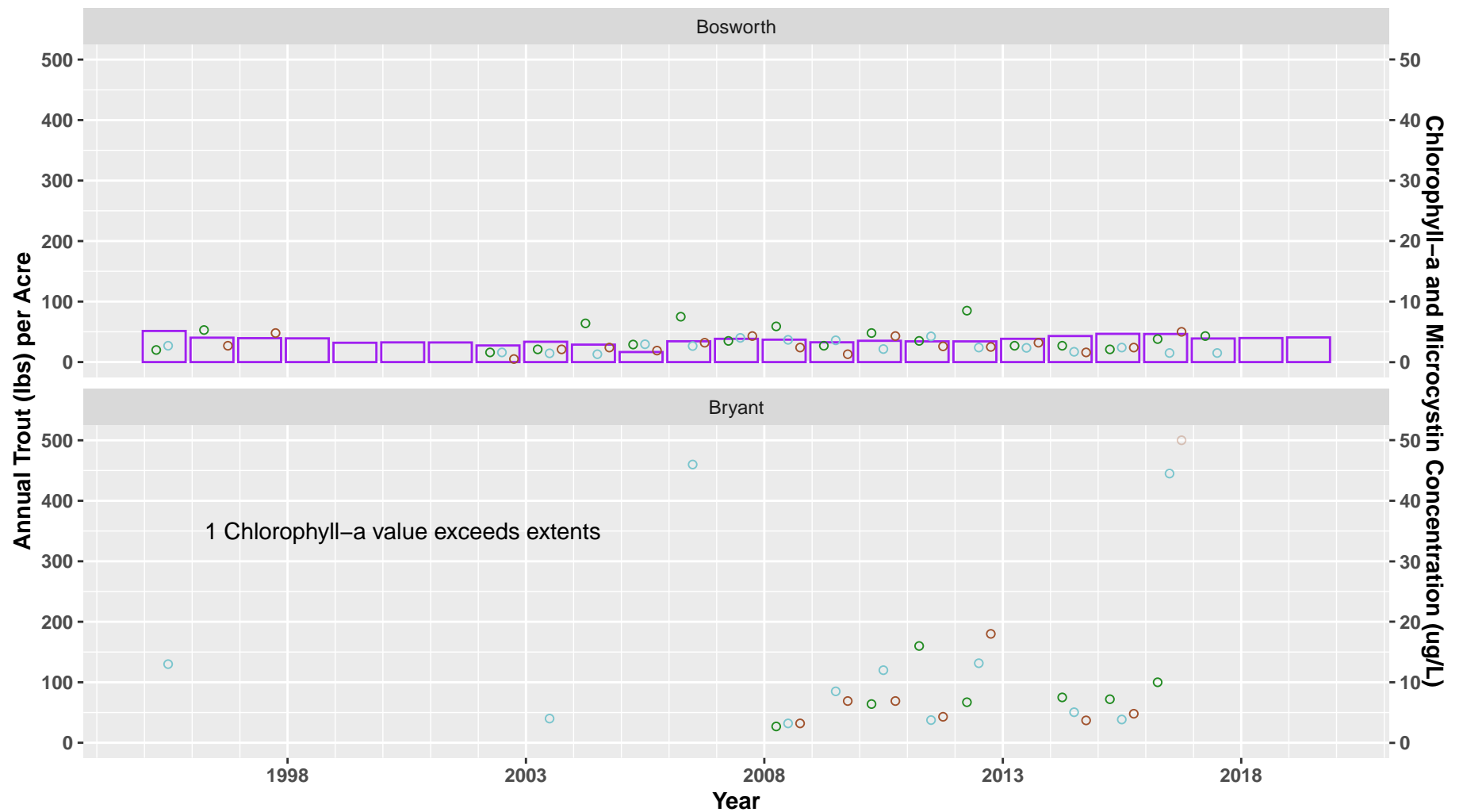


Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

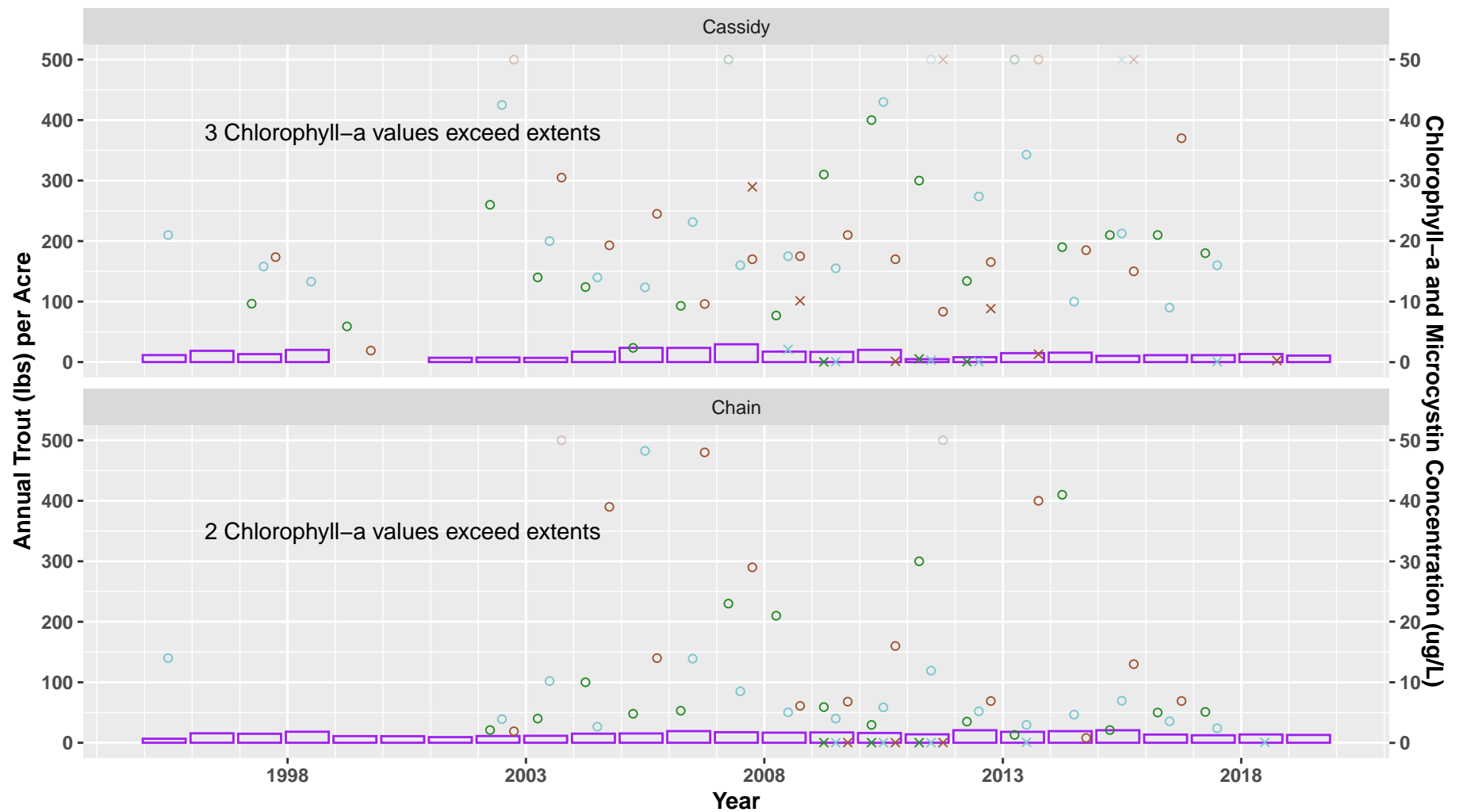




Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Trout lbs per Acre

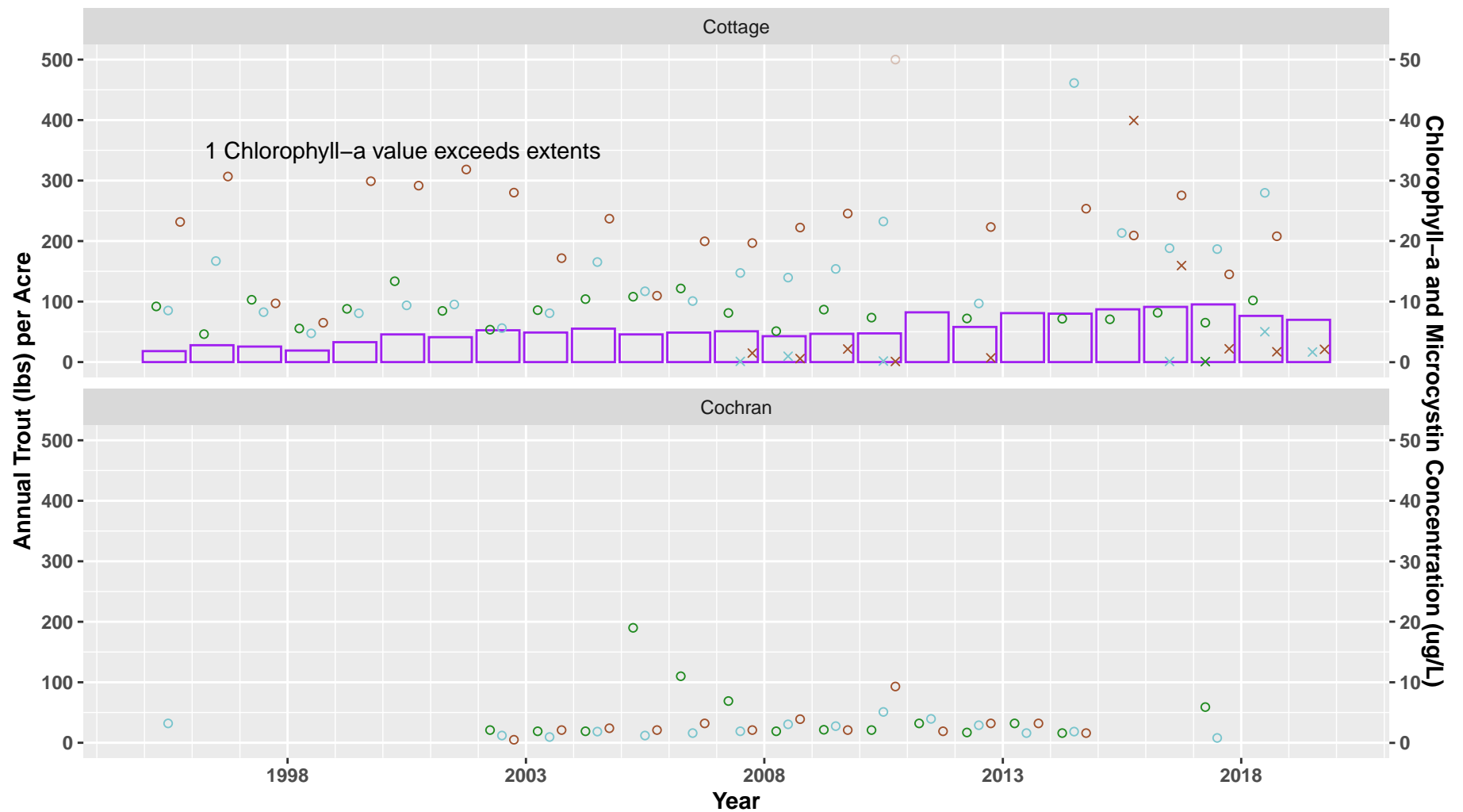
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

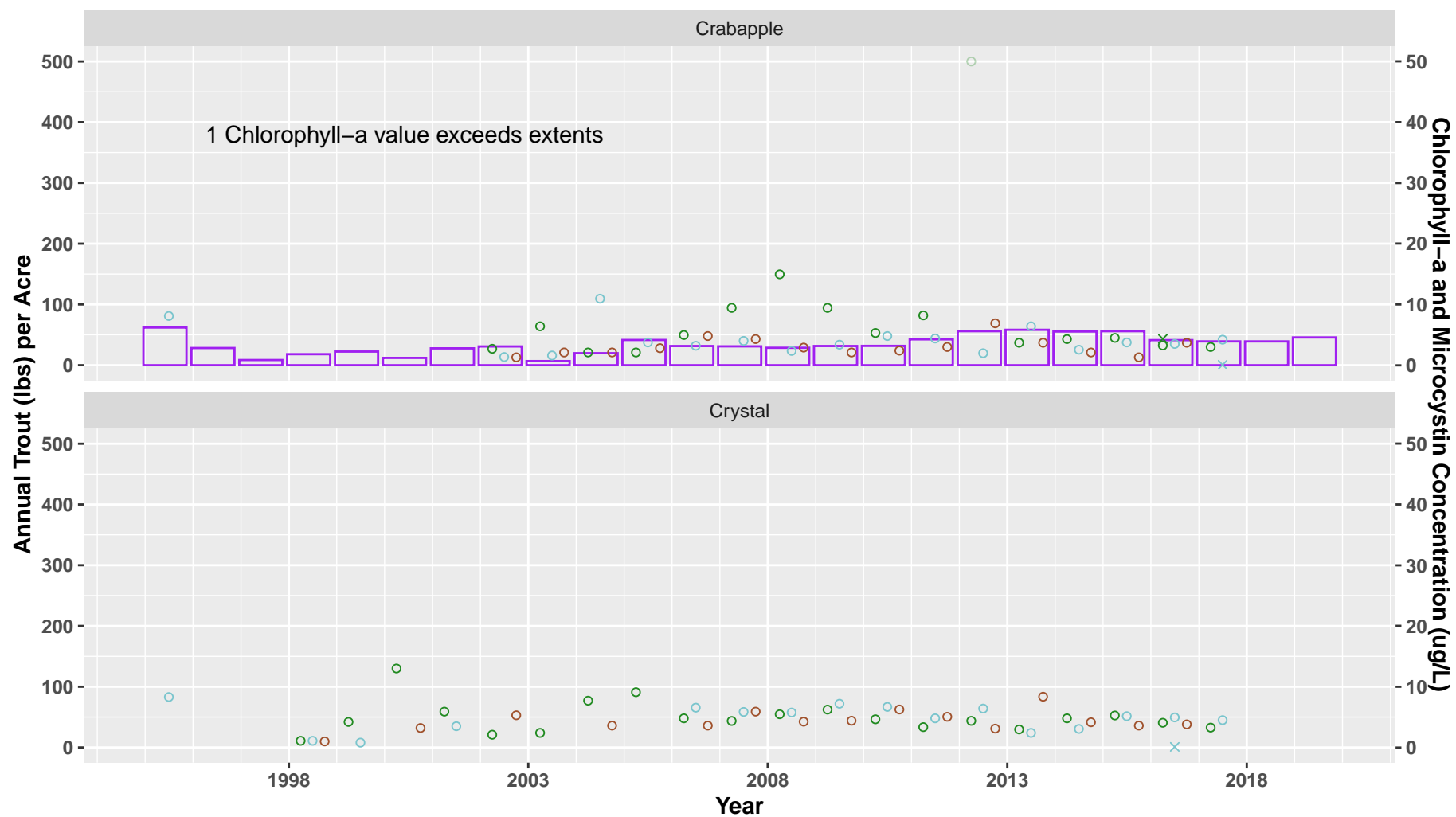
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

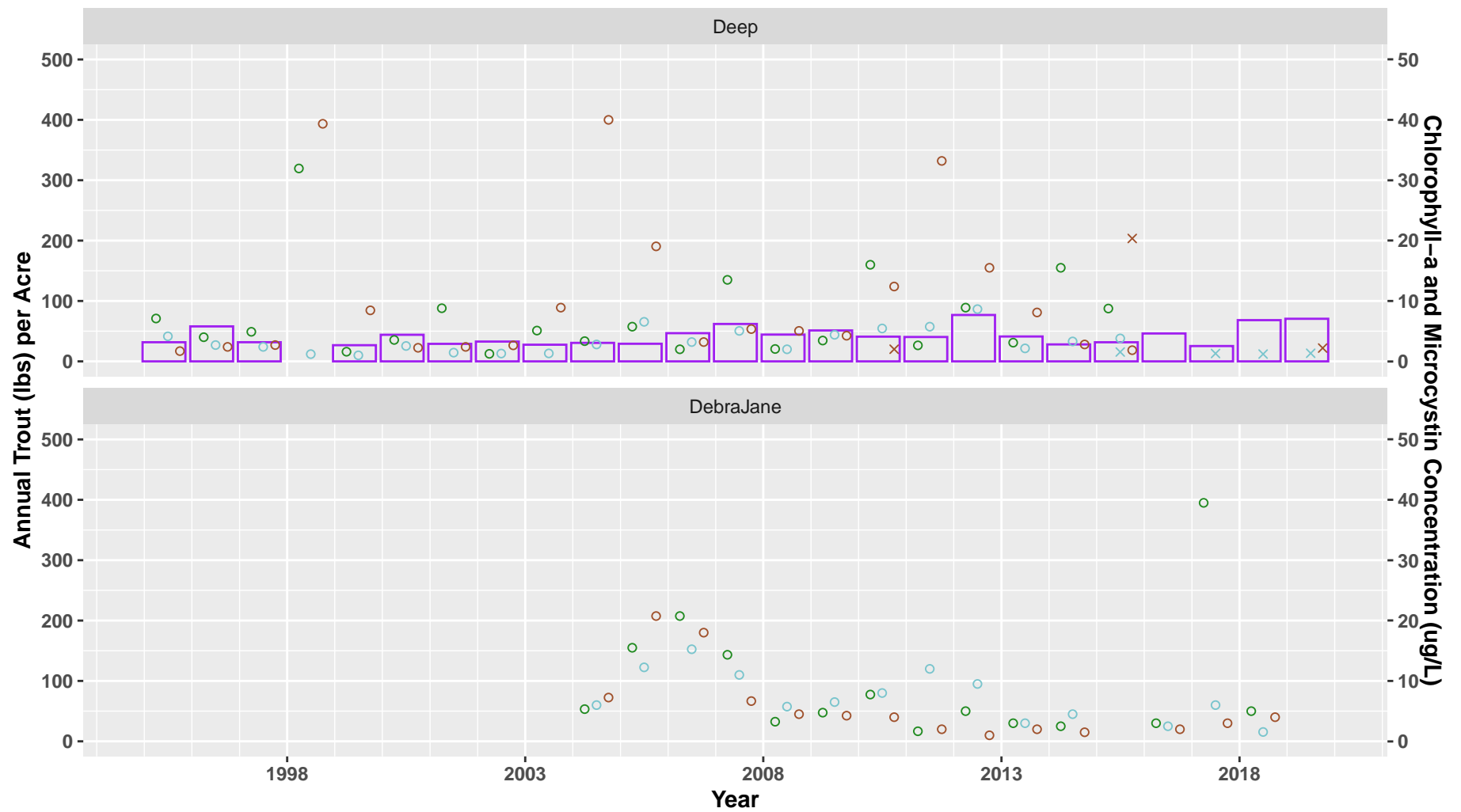
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

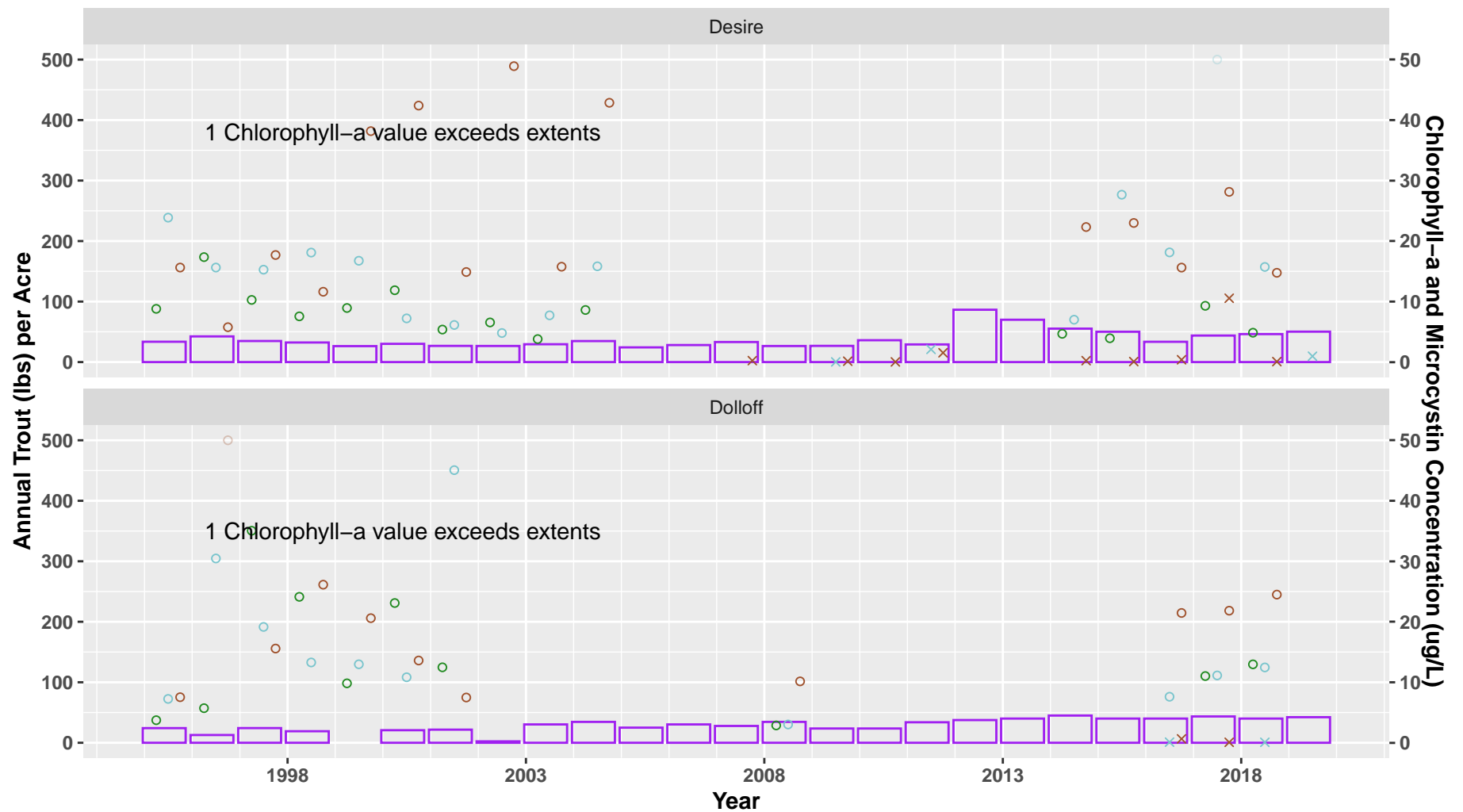
Season ● Spring ● Summer ● Fall



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

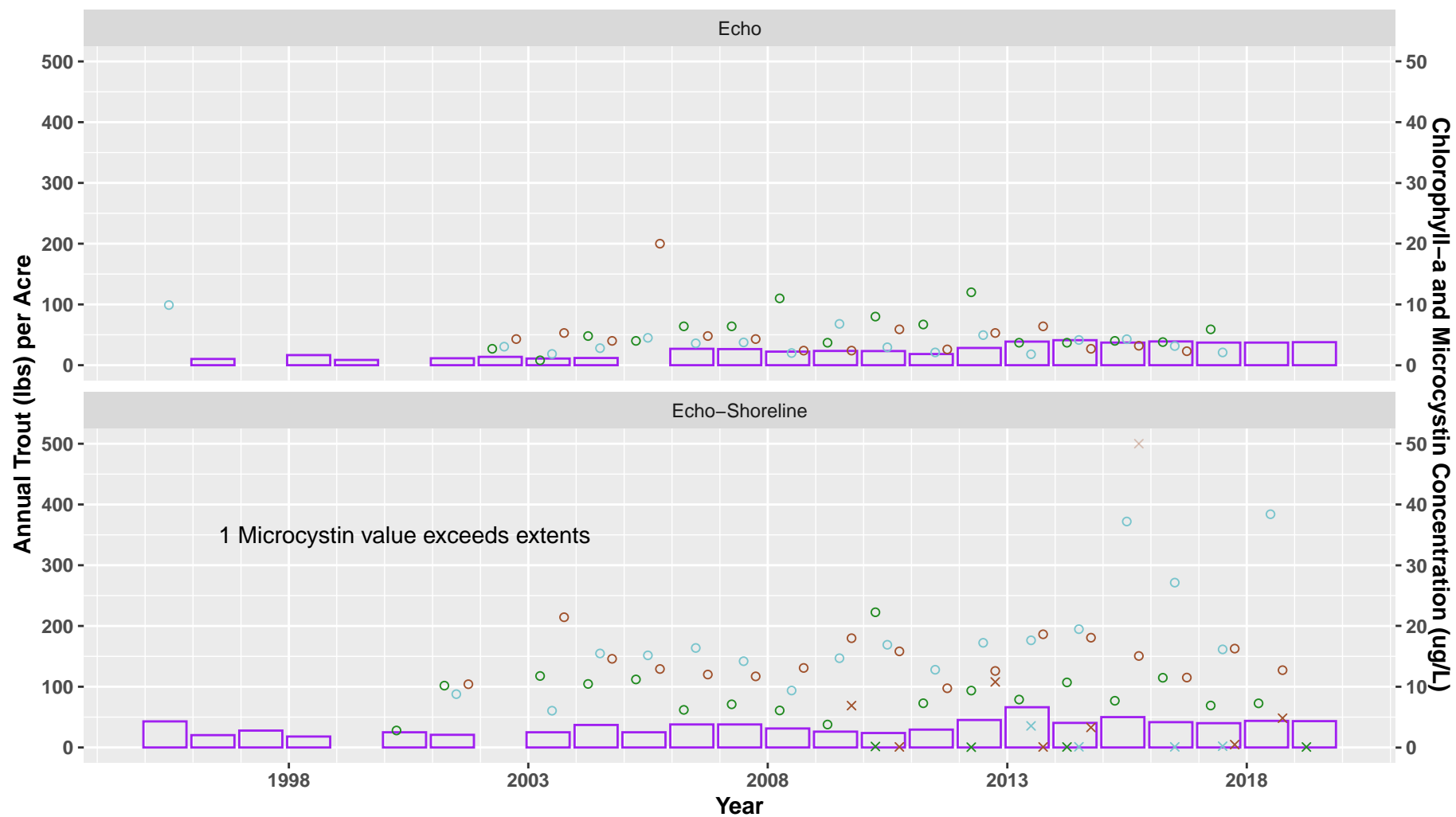
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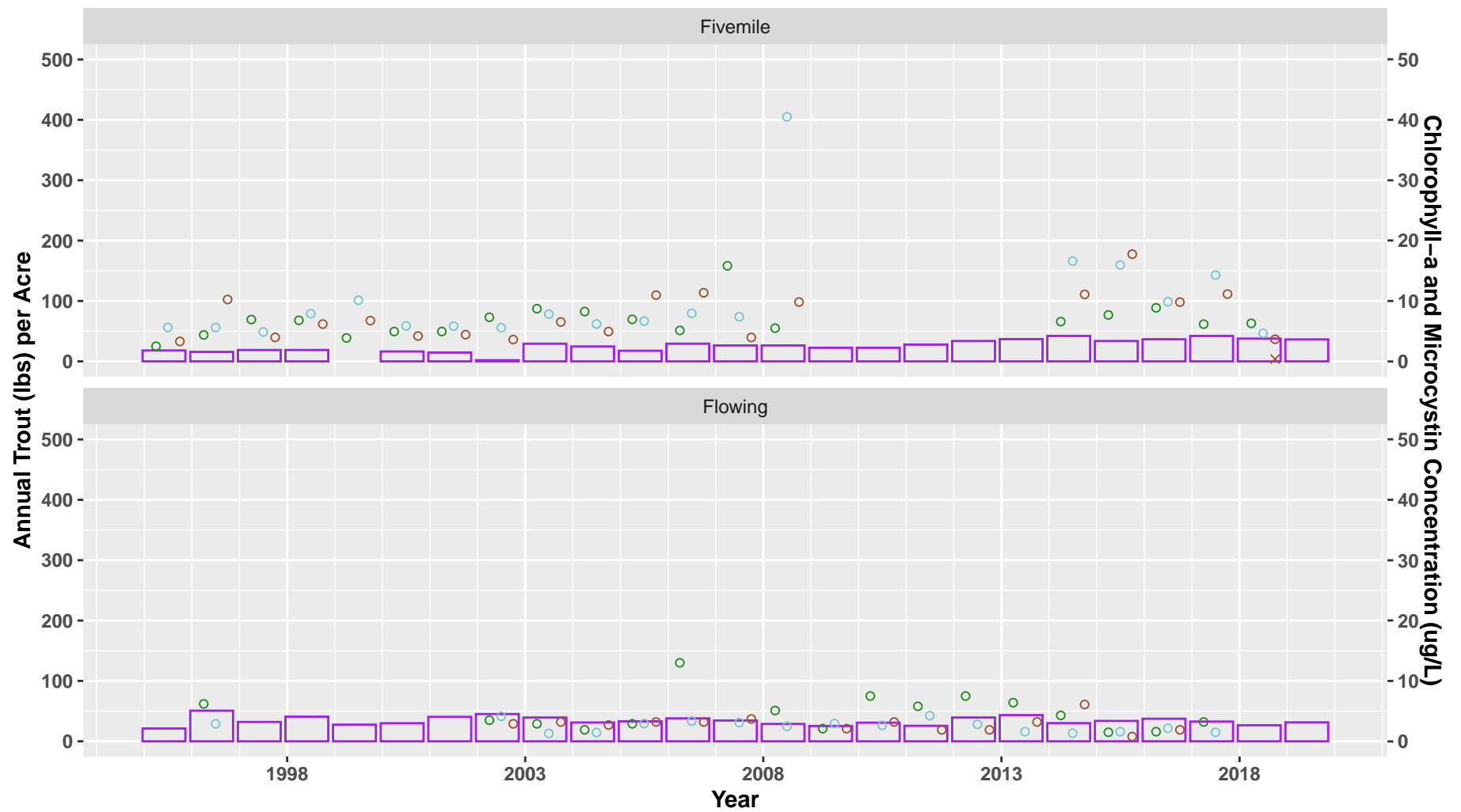


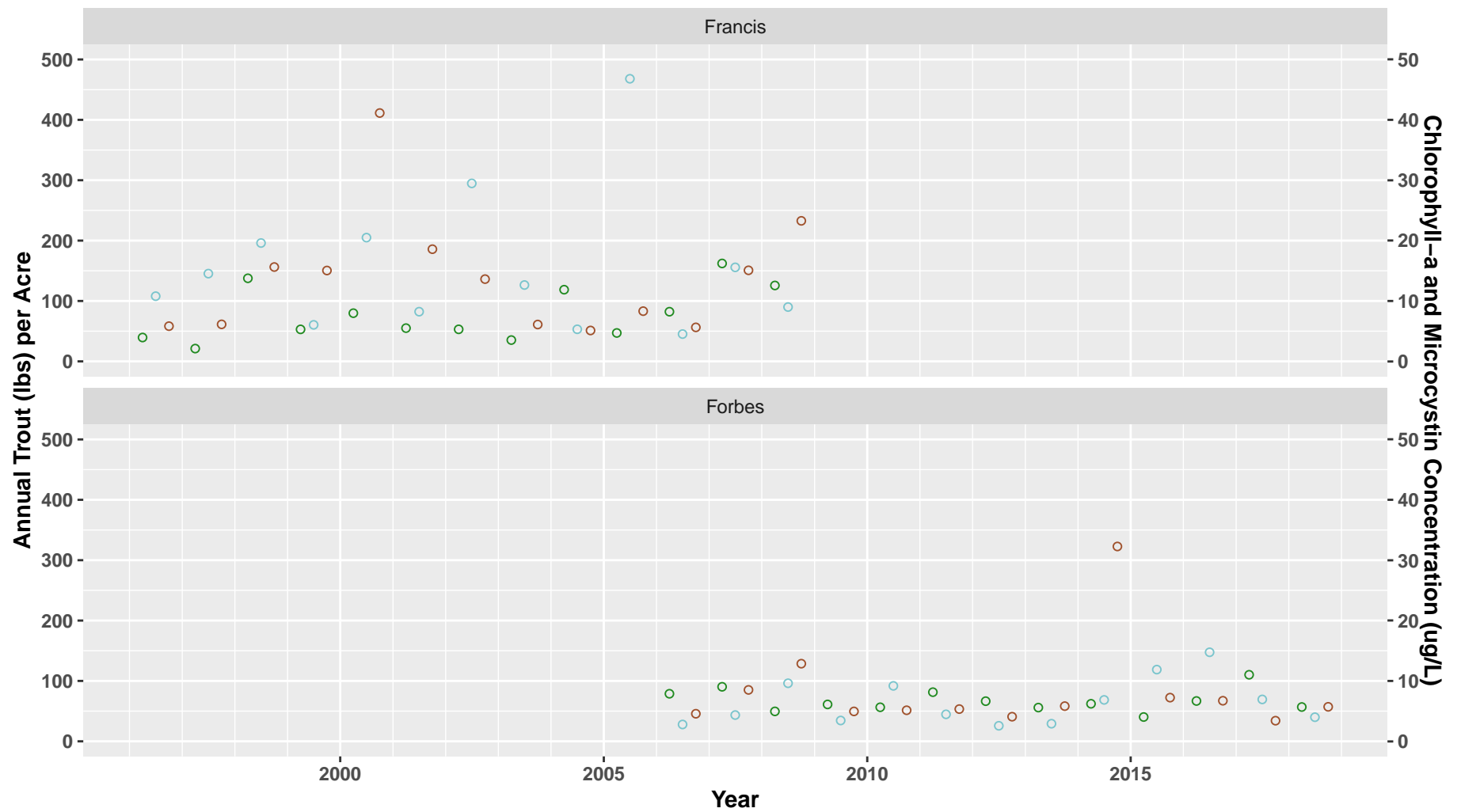
Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall



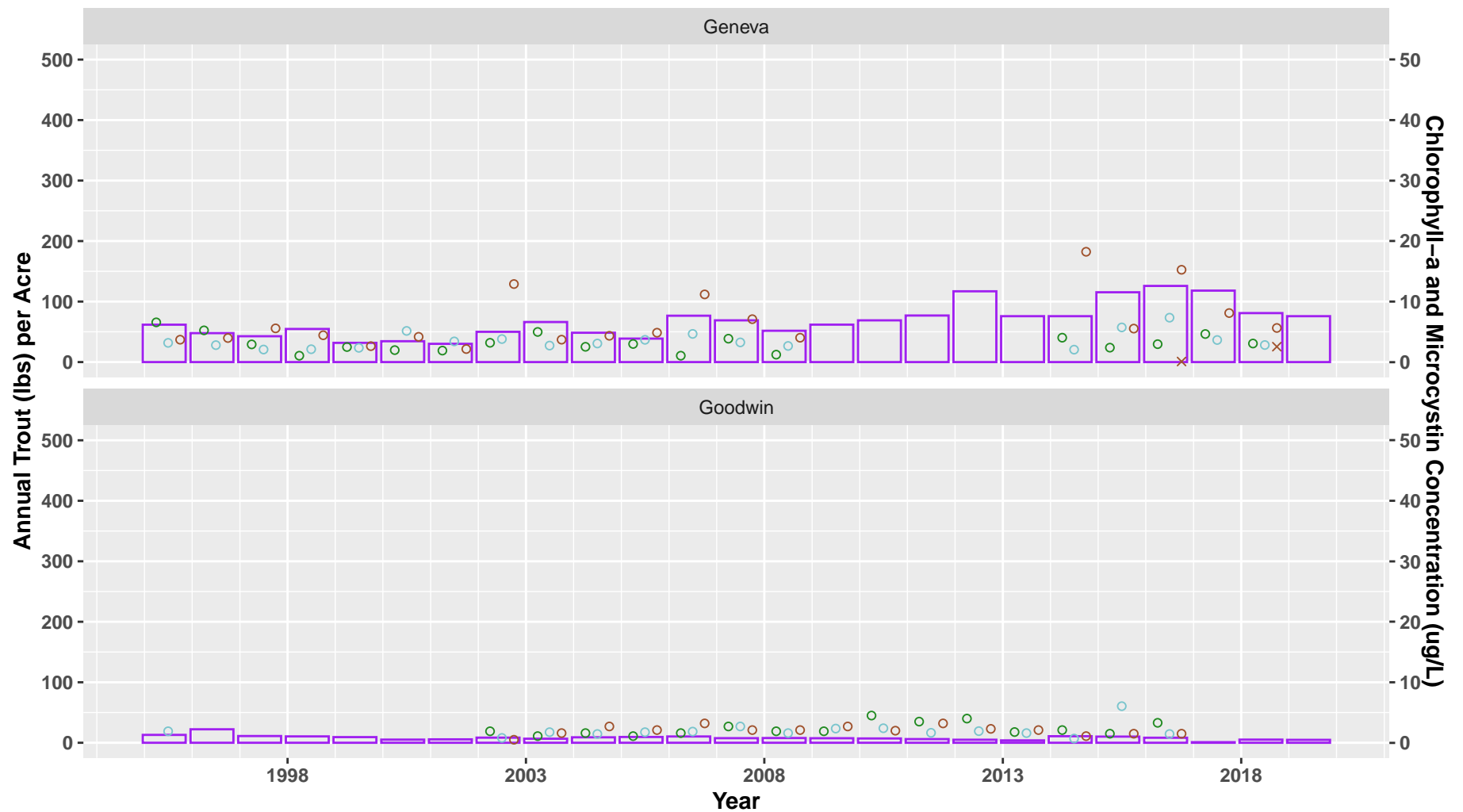


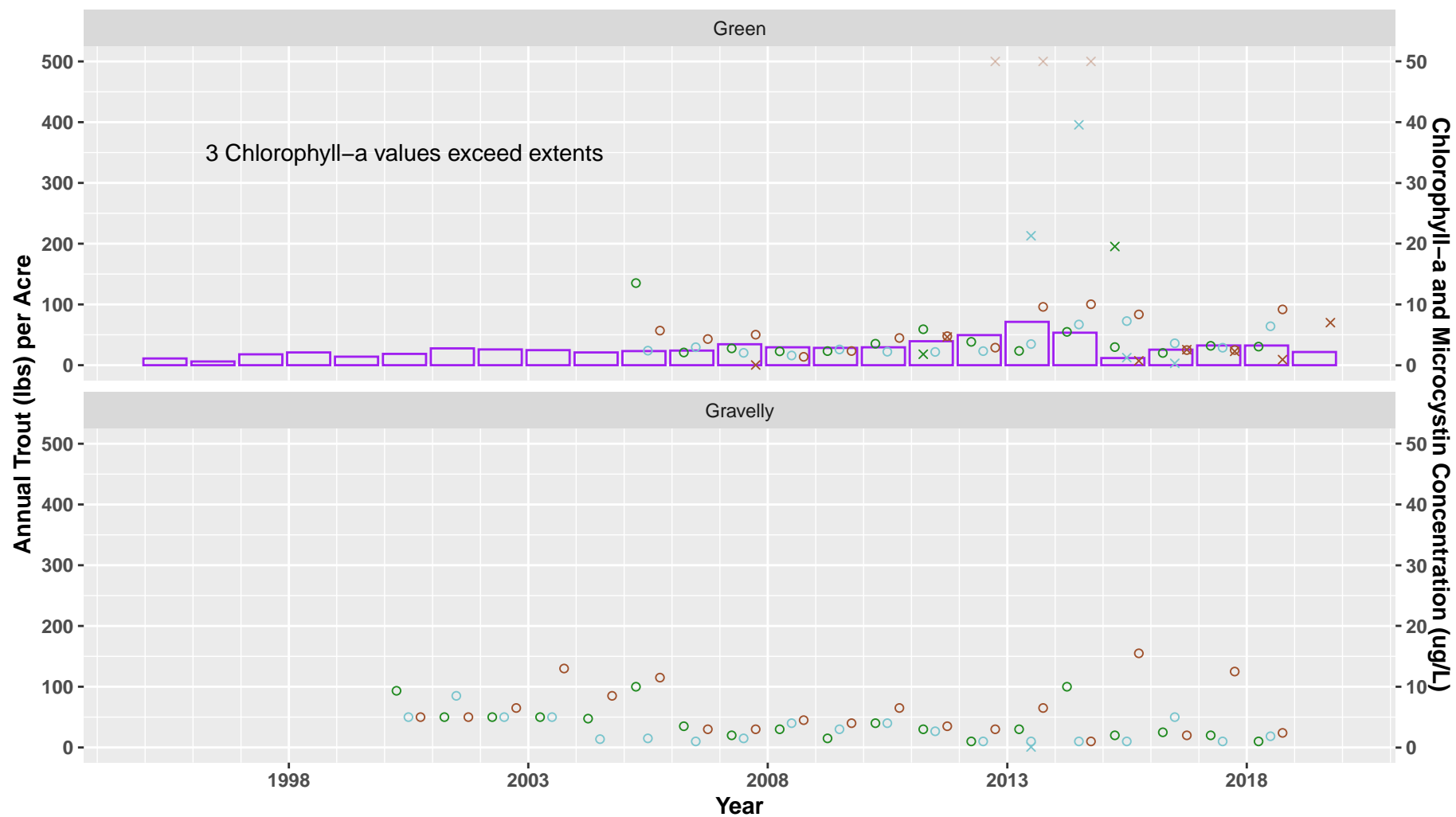


Parameter ○ Chlorophyll-a × Trout lbs per Acre

Season ● Spring ● Summer ● Fall

Shading ● within extents

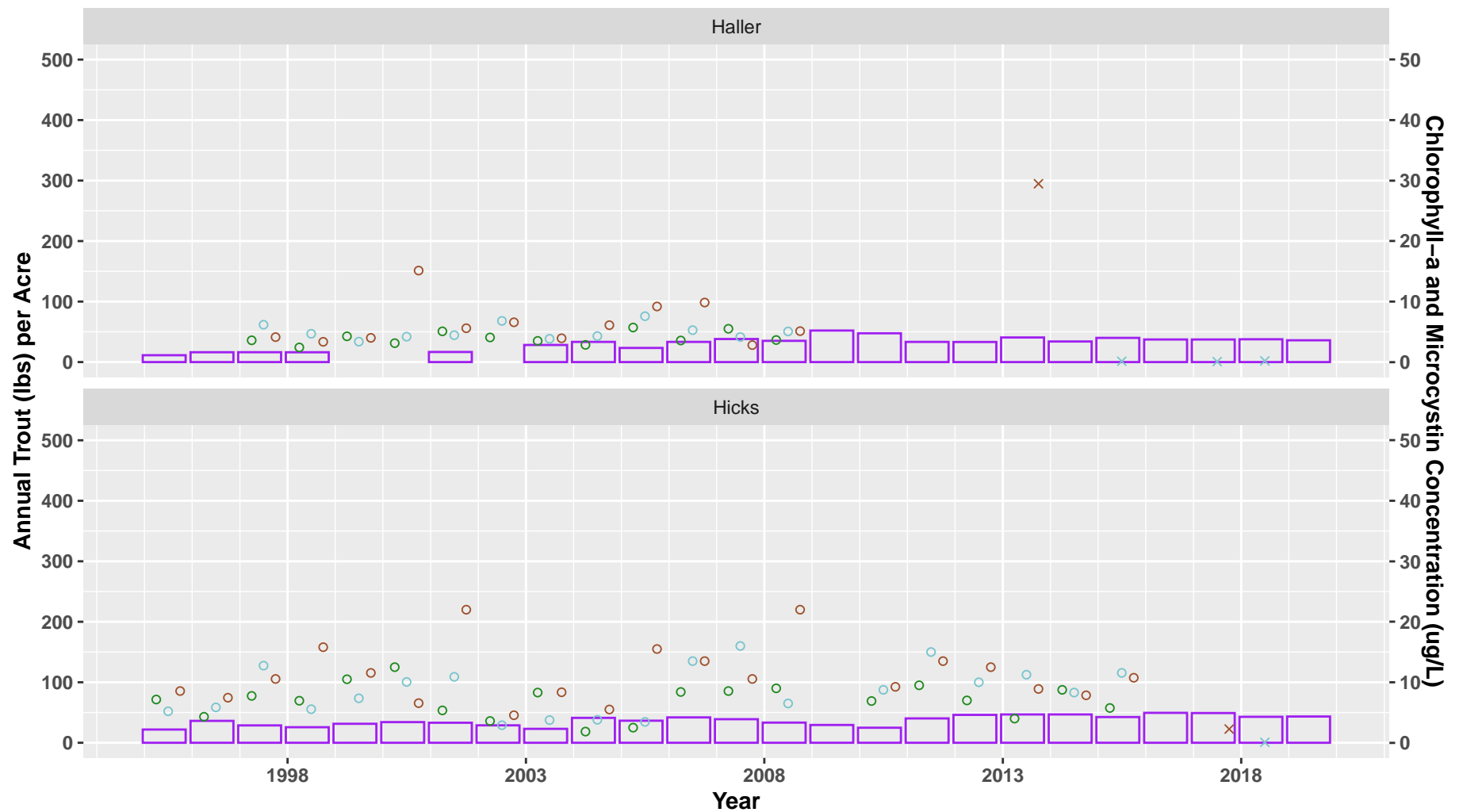




Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

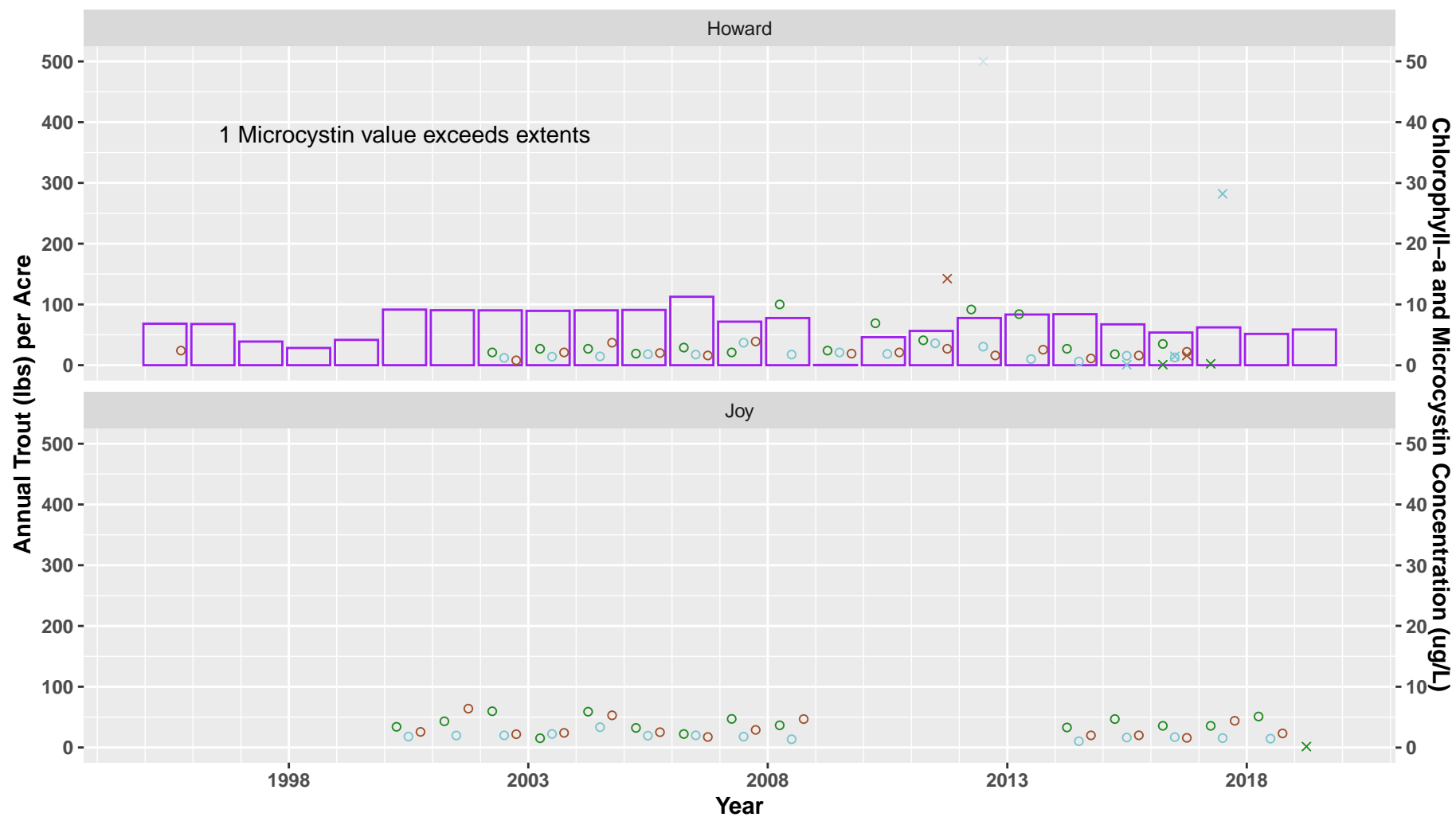
Season ● Spring ● Summer ● Fall



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

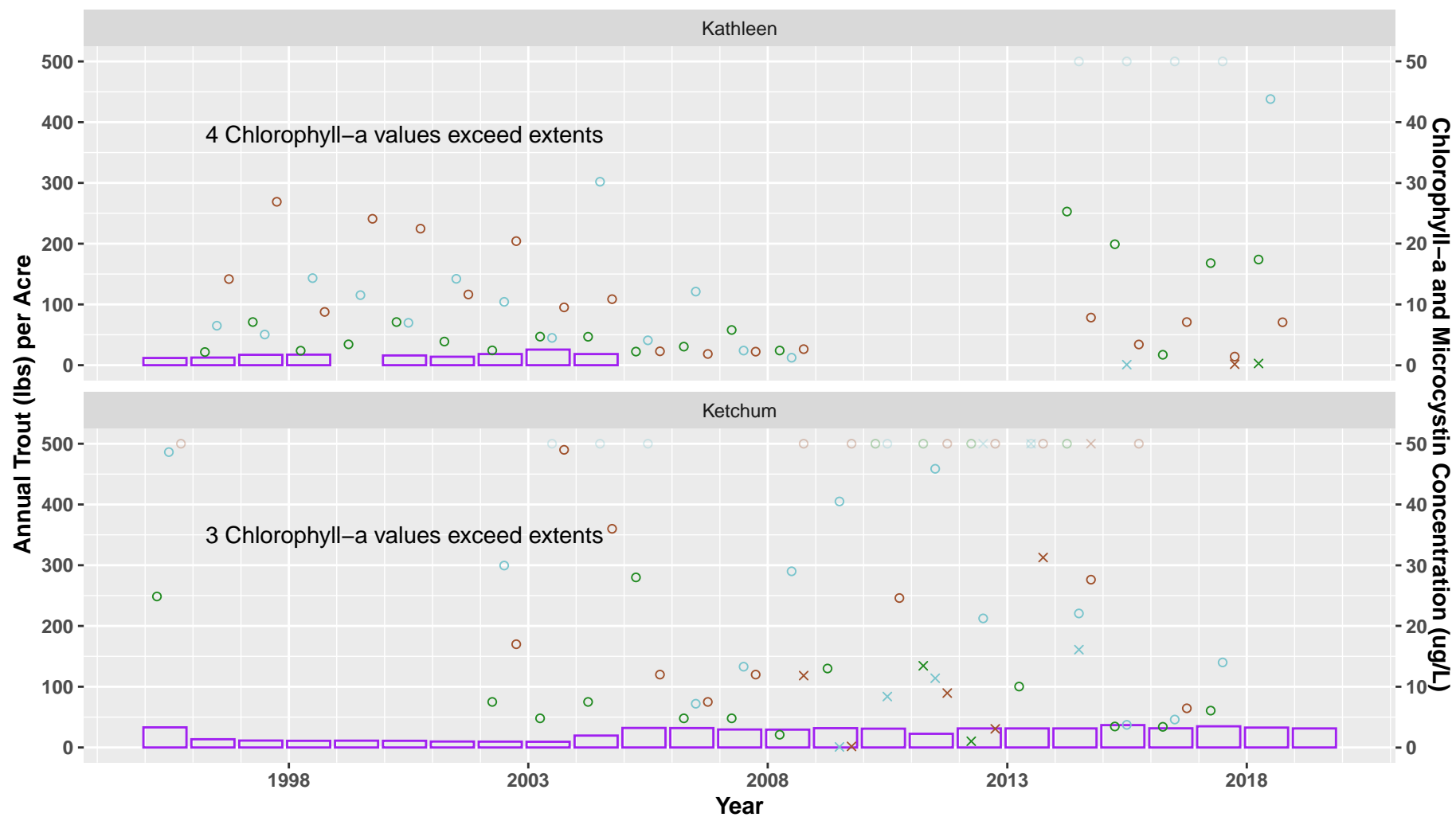
Shading ● within extents



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

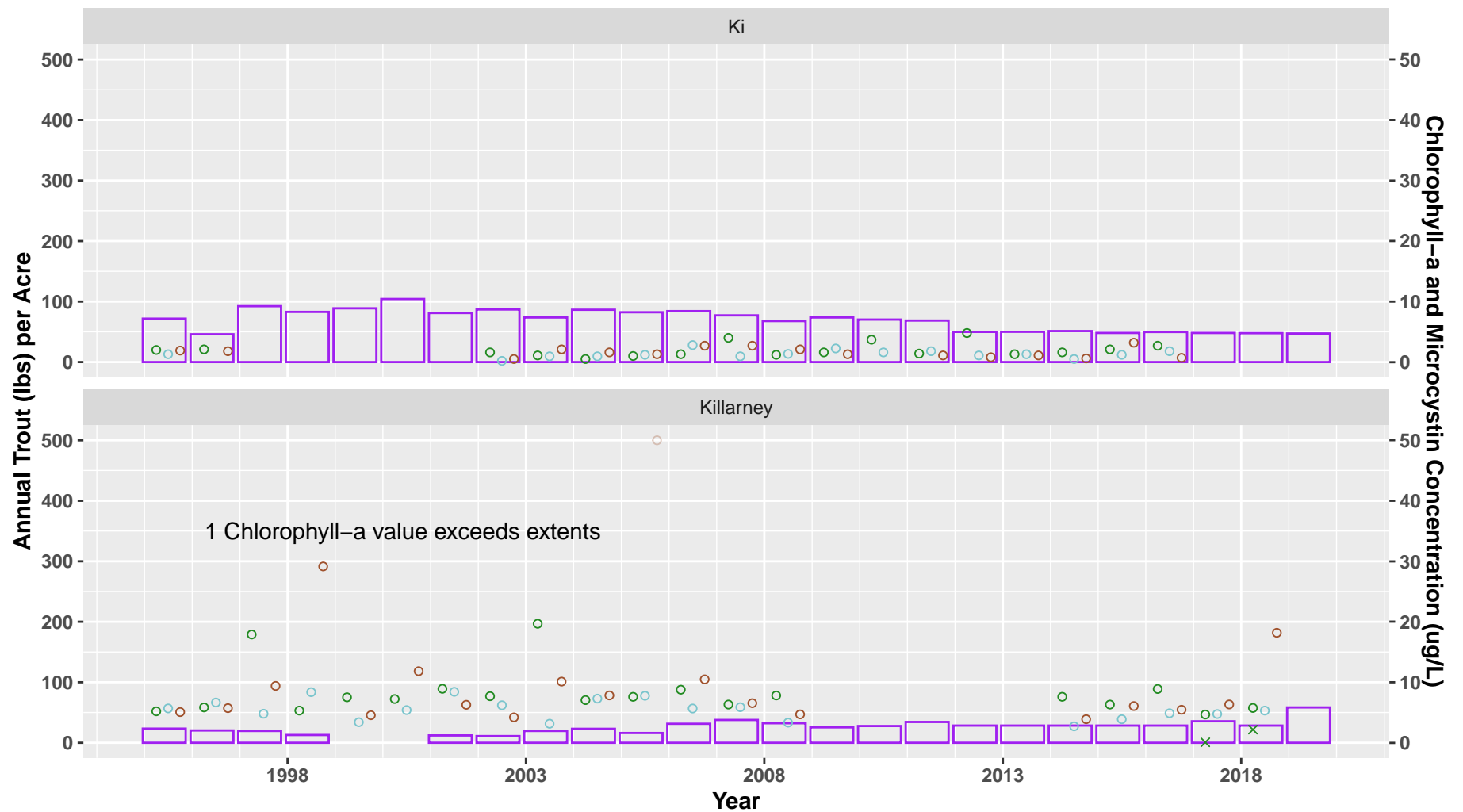
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

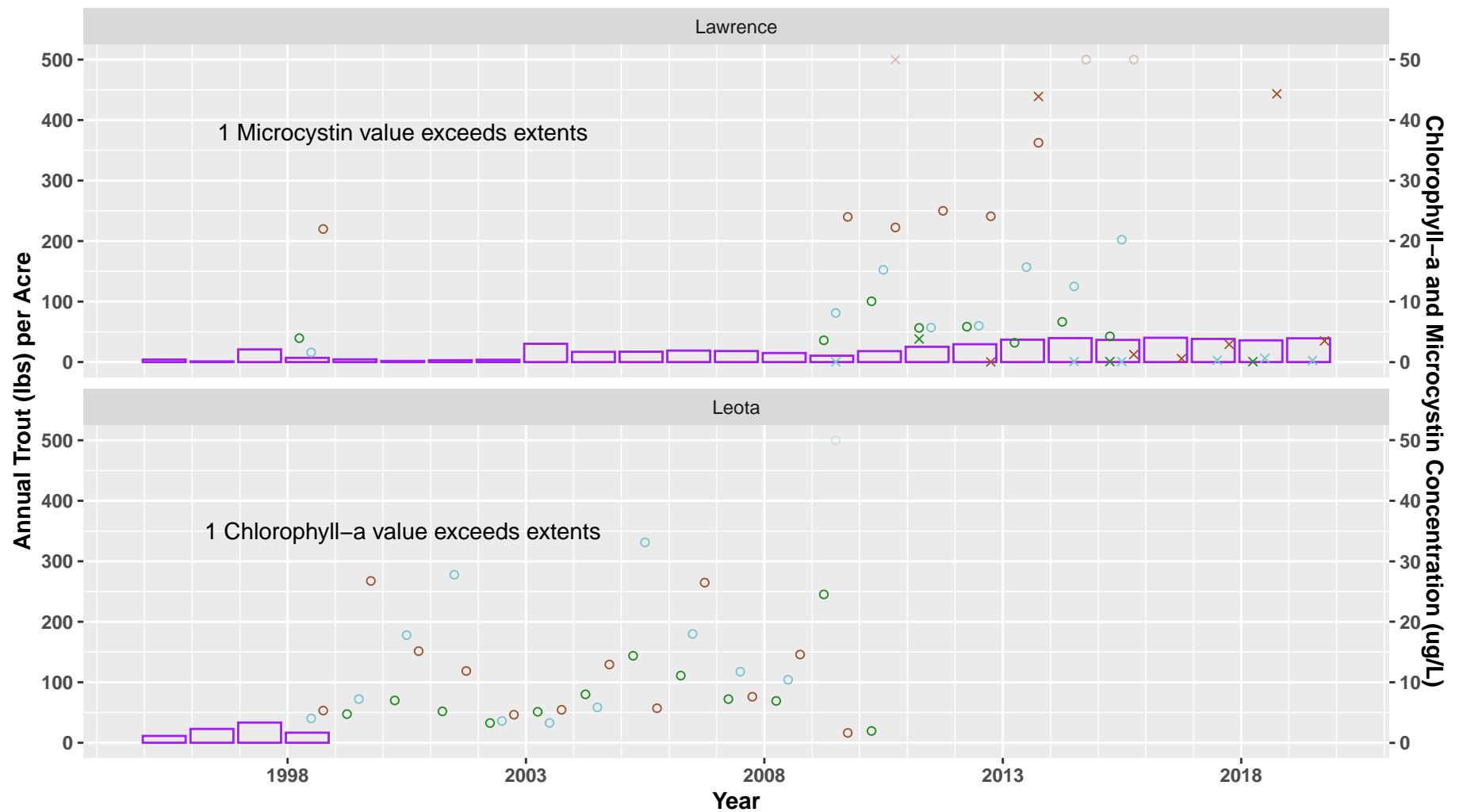
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

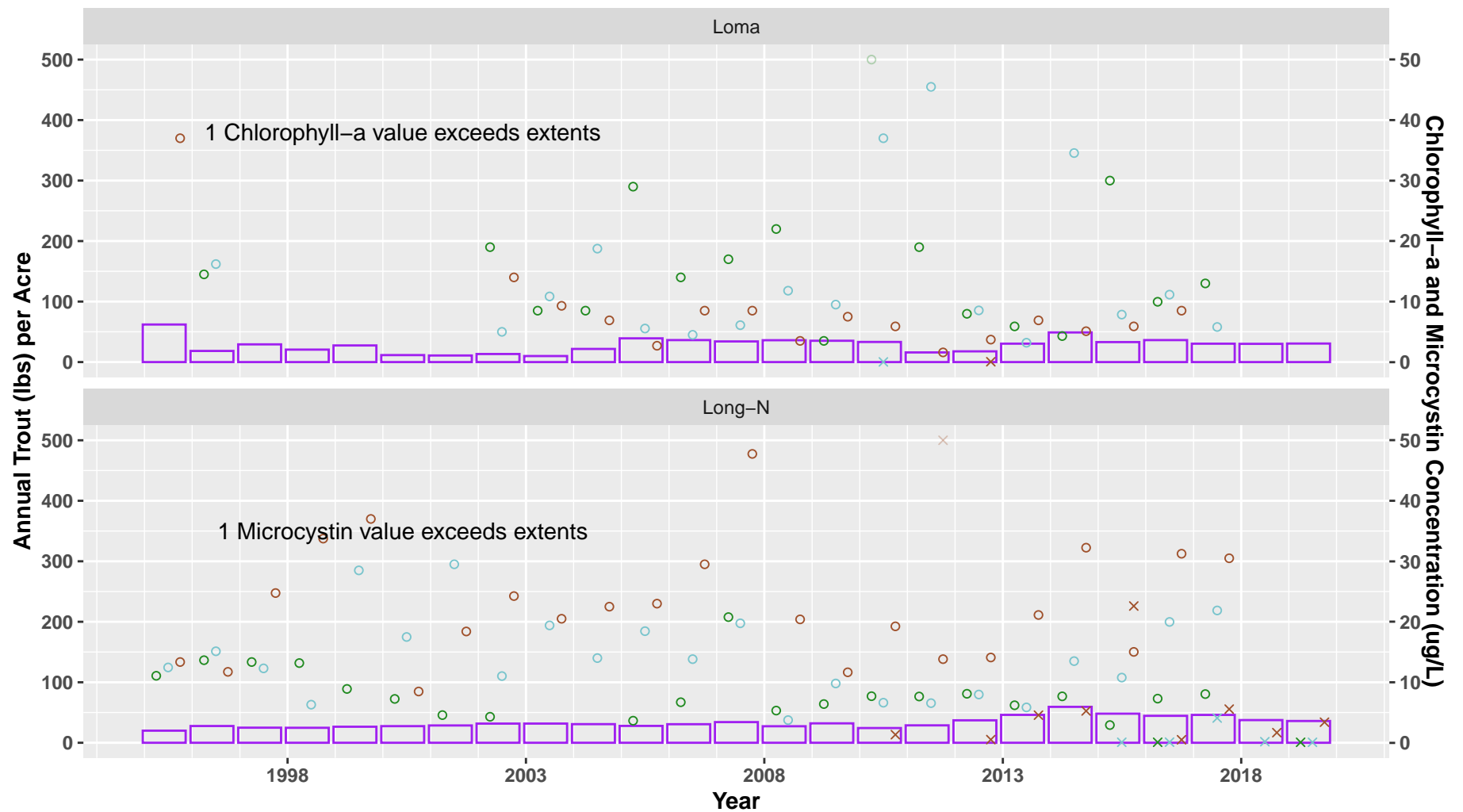
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

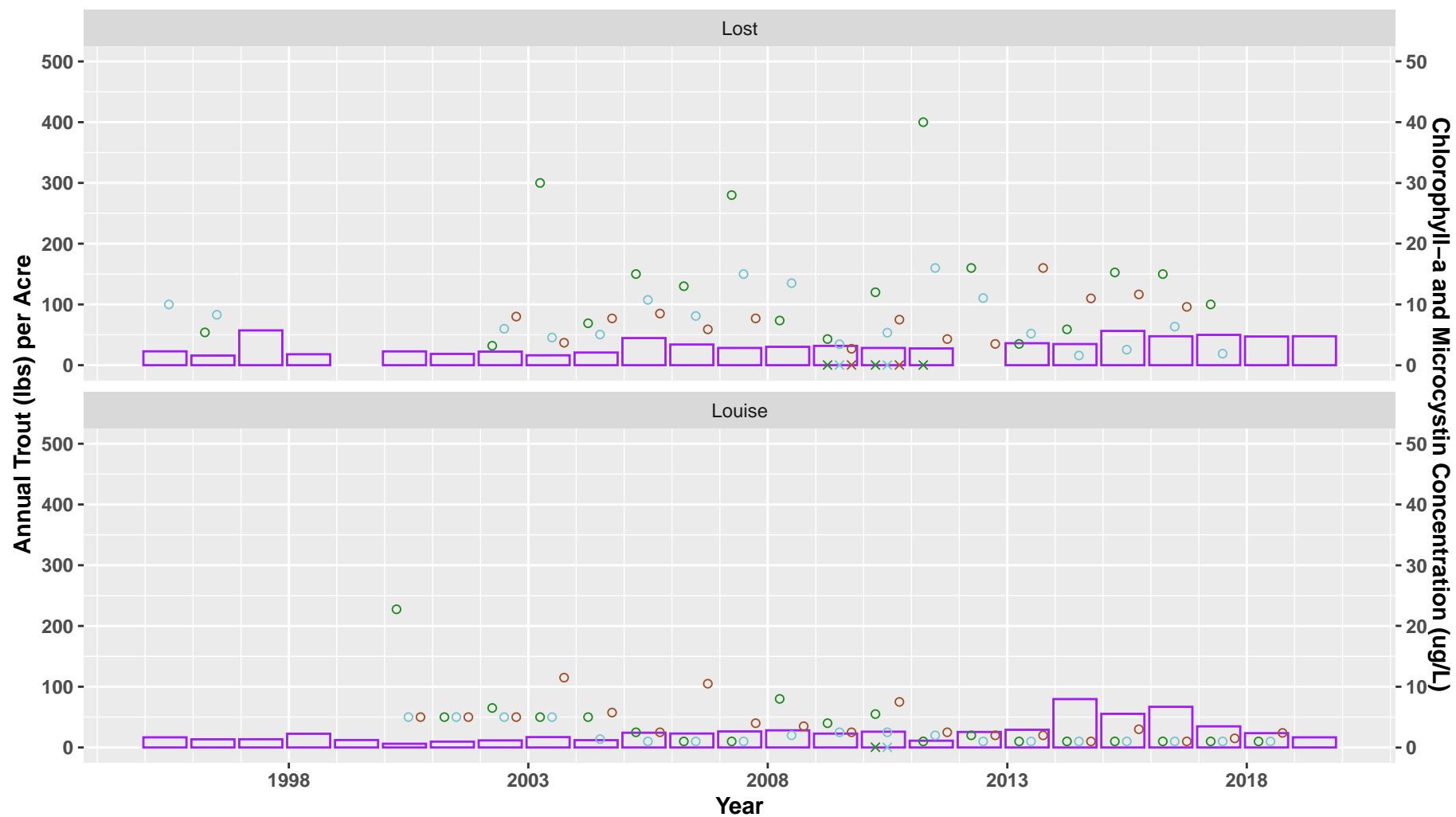
Season ● Spring ● Summer ● Fall



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

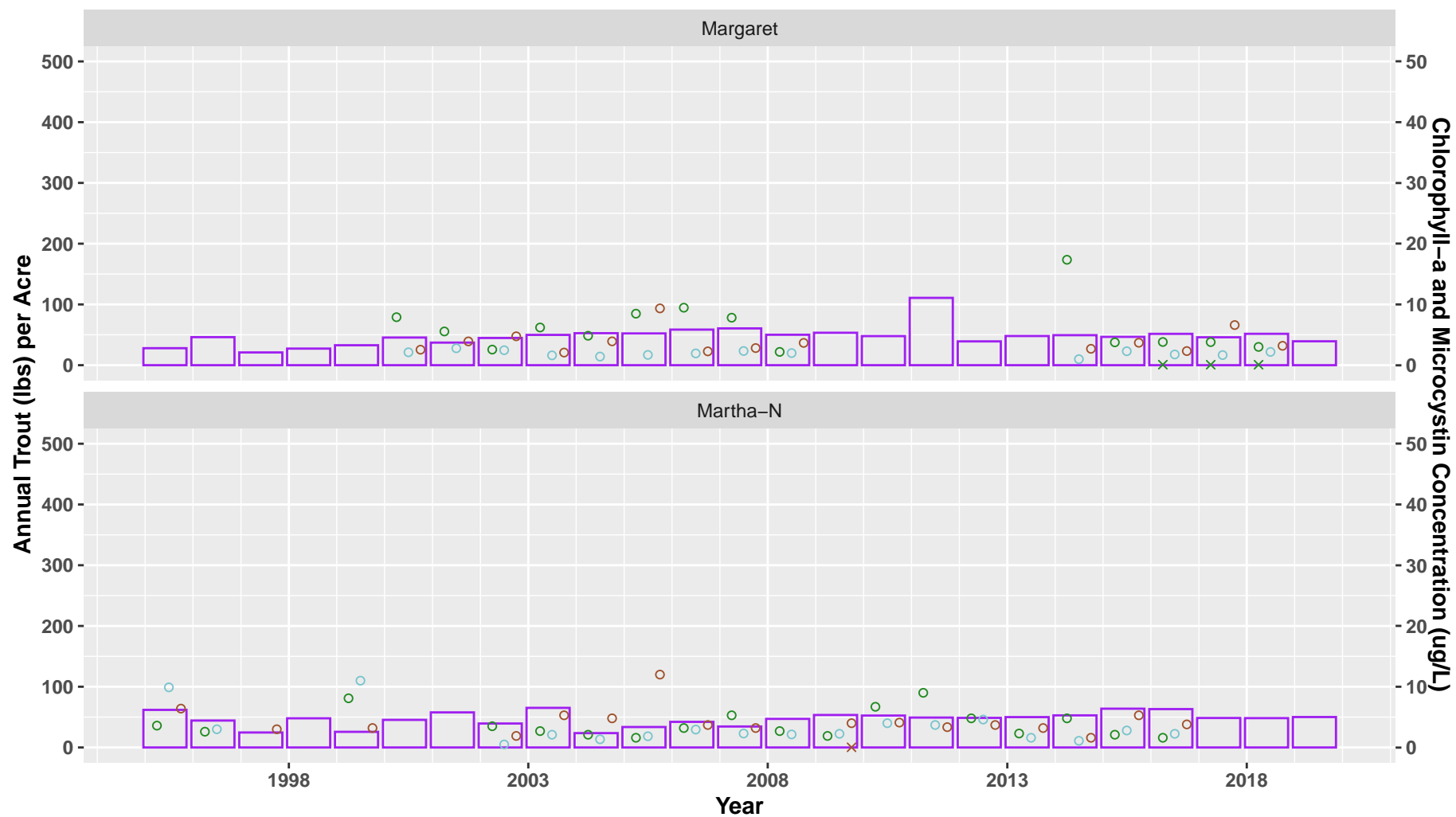
Shading ● within extents

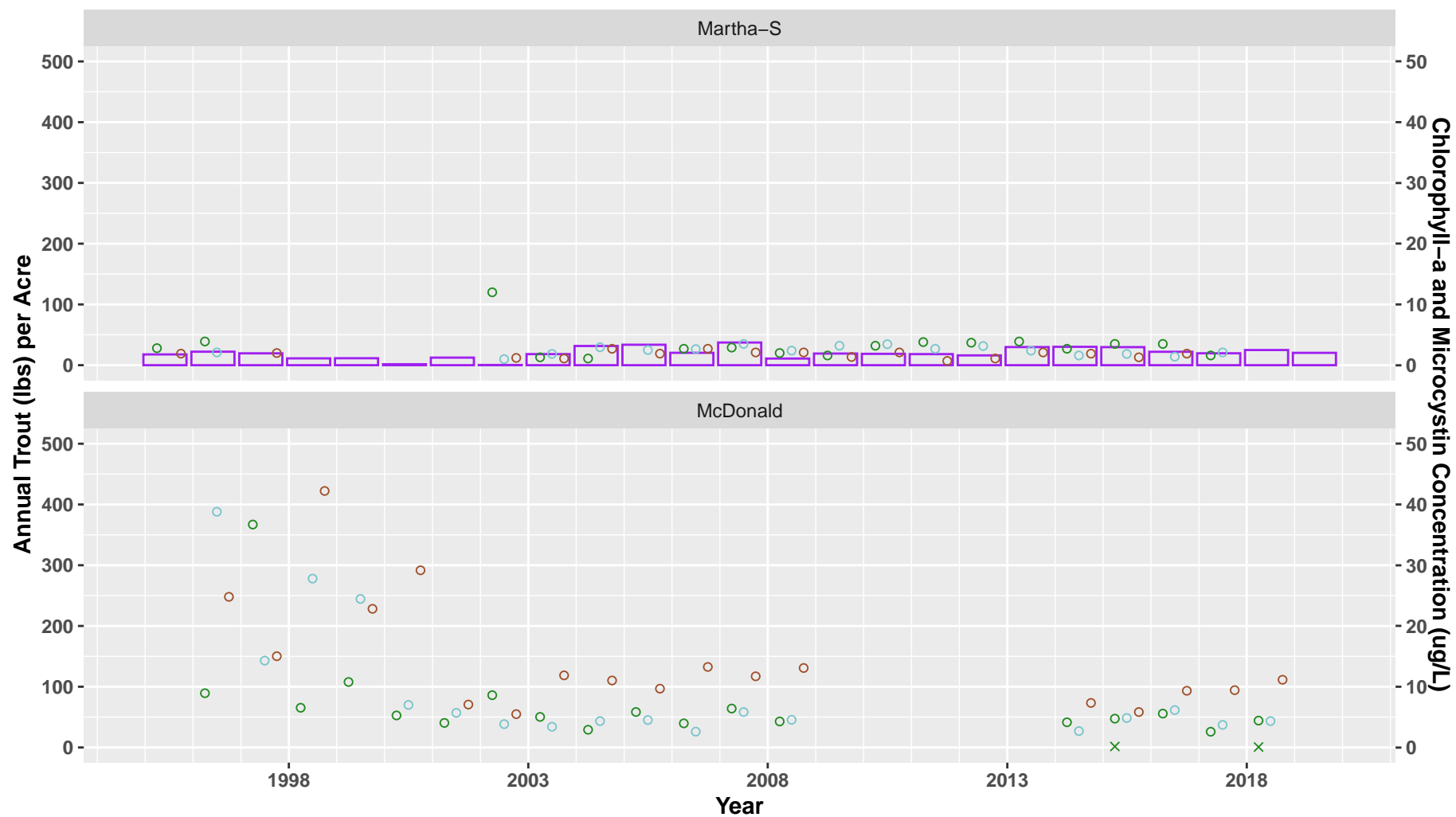


Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

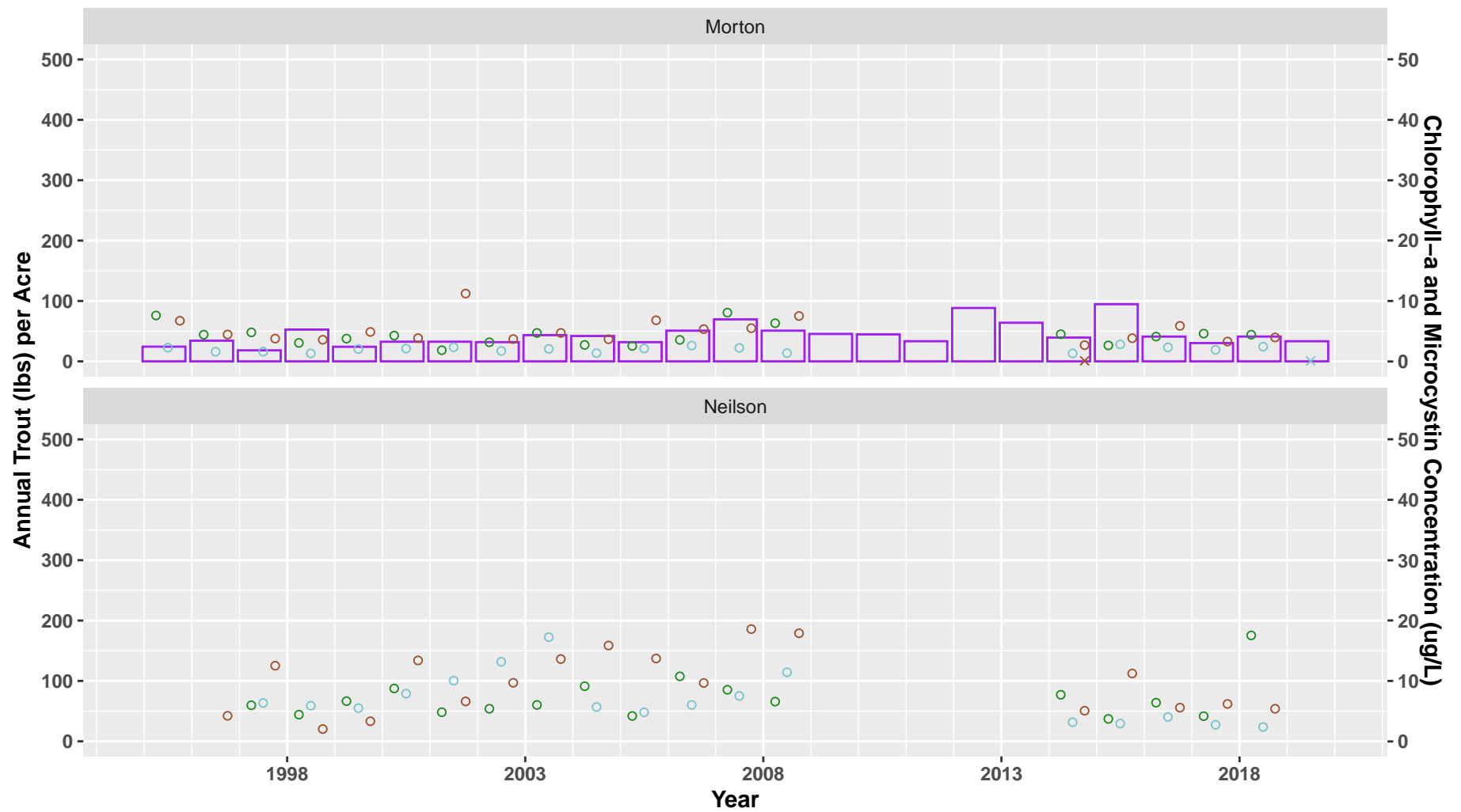




Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

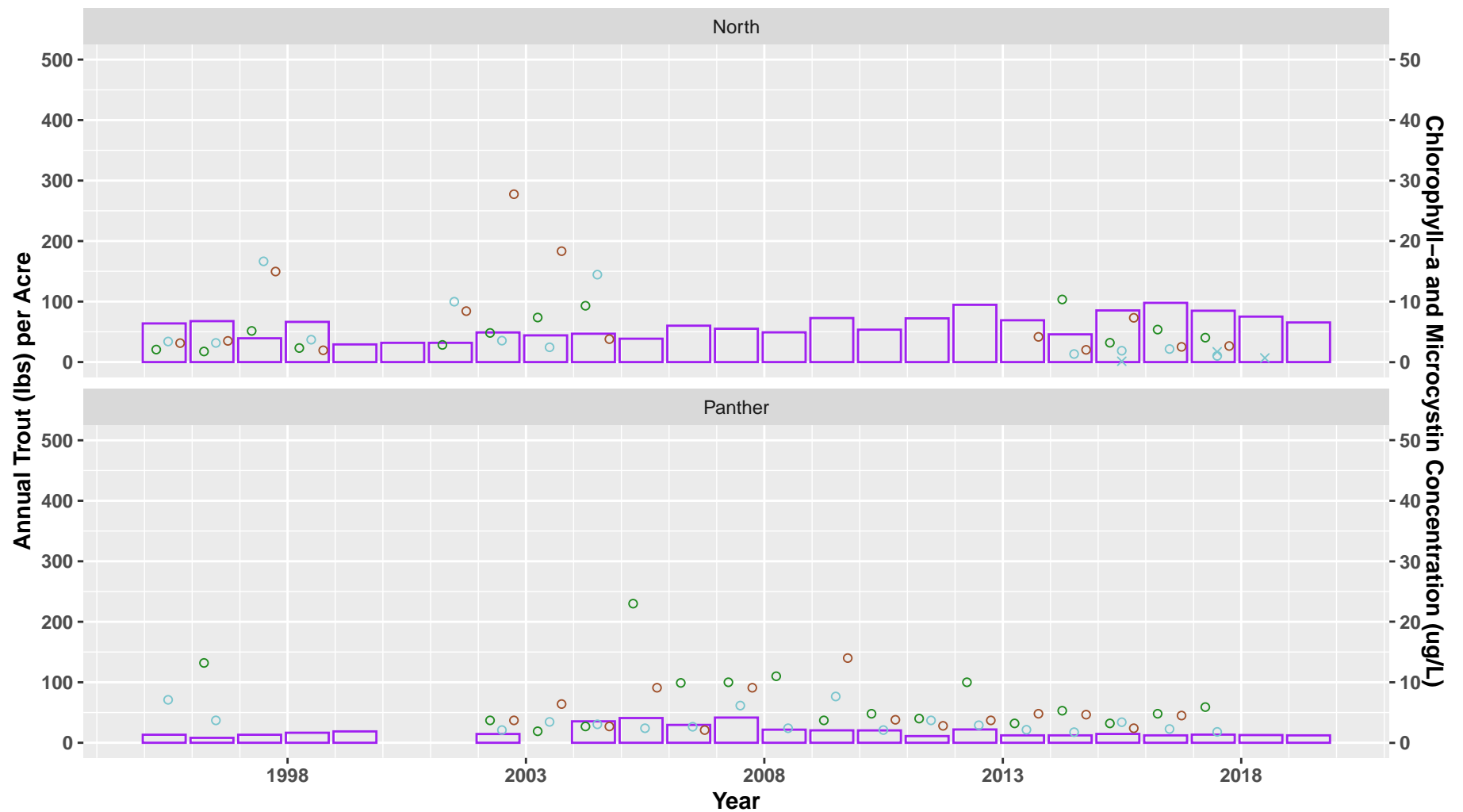
Shading ● within extents



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

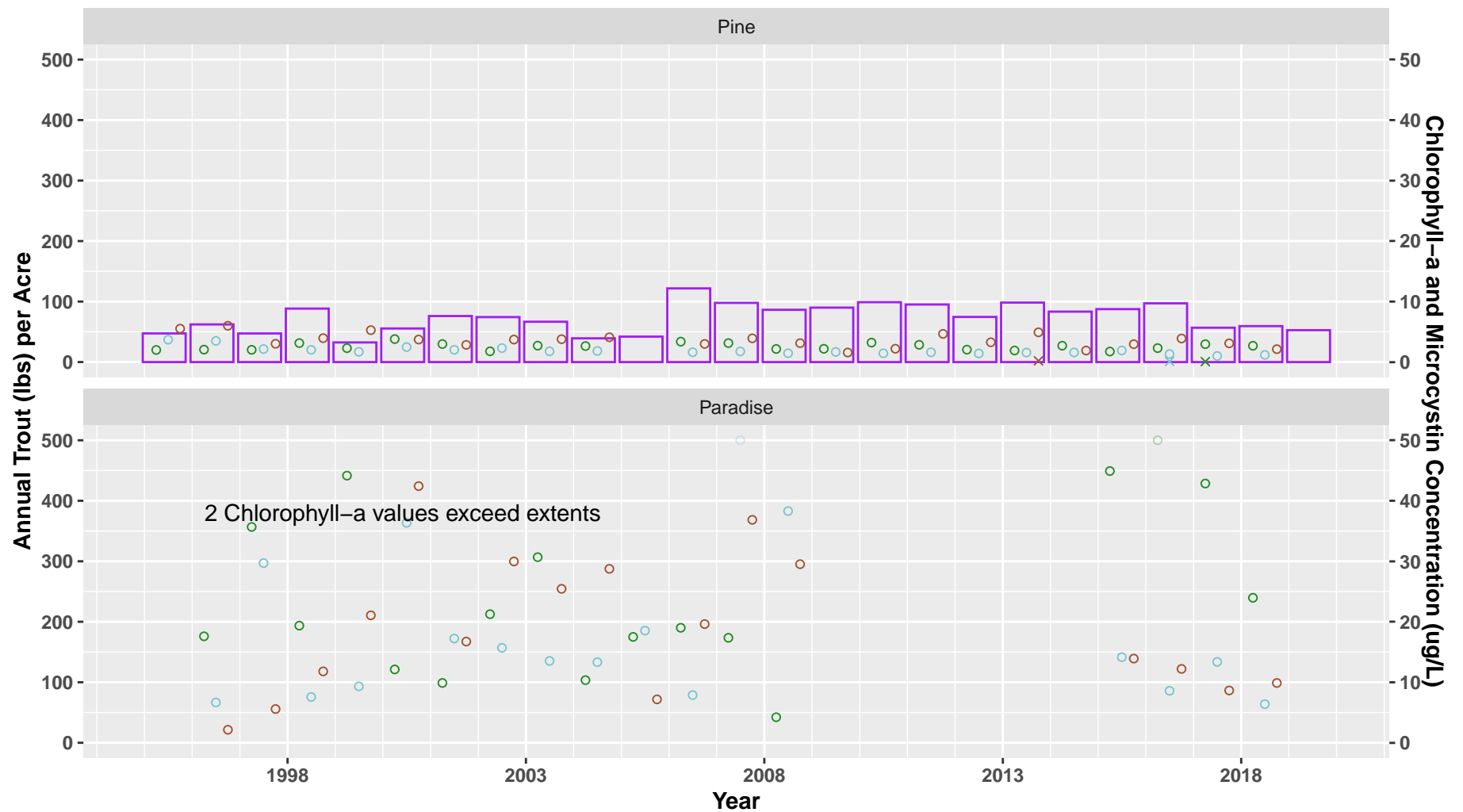
Shading ● within extents



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

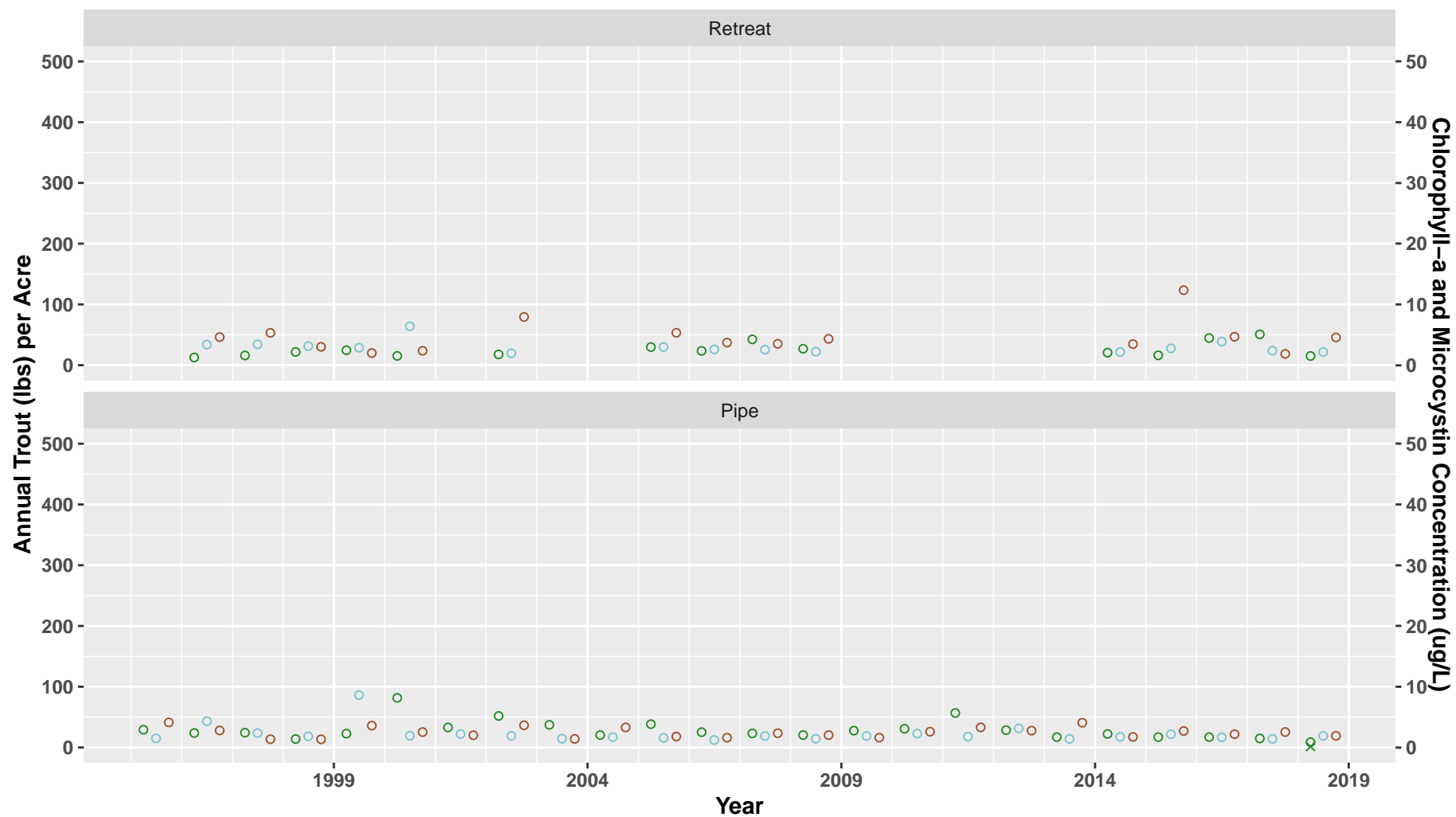
Shading ● within extents



Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

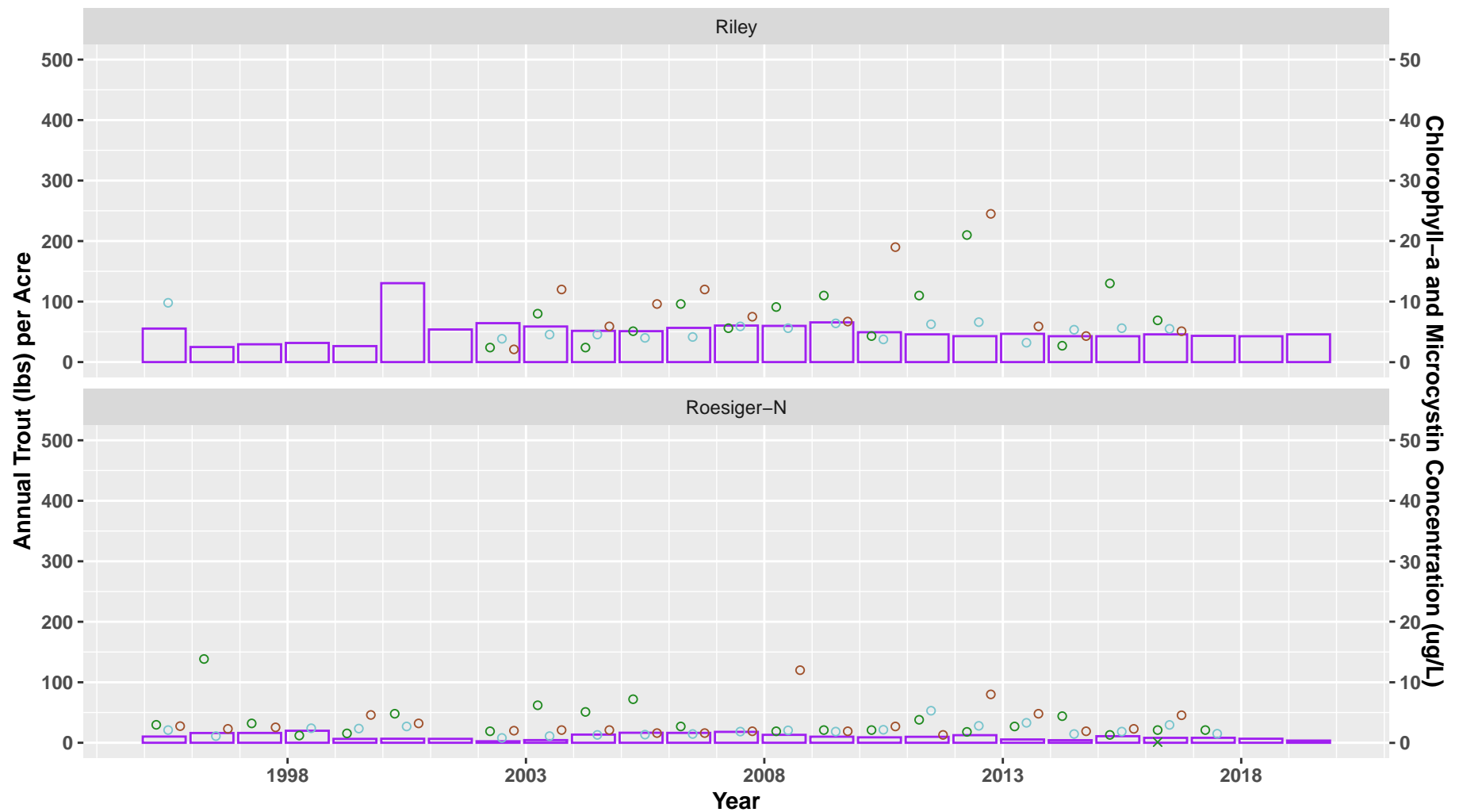
Season ● Spring ● Summer ● Fall

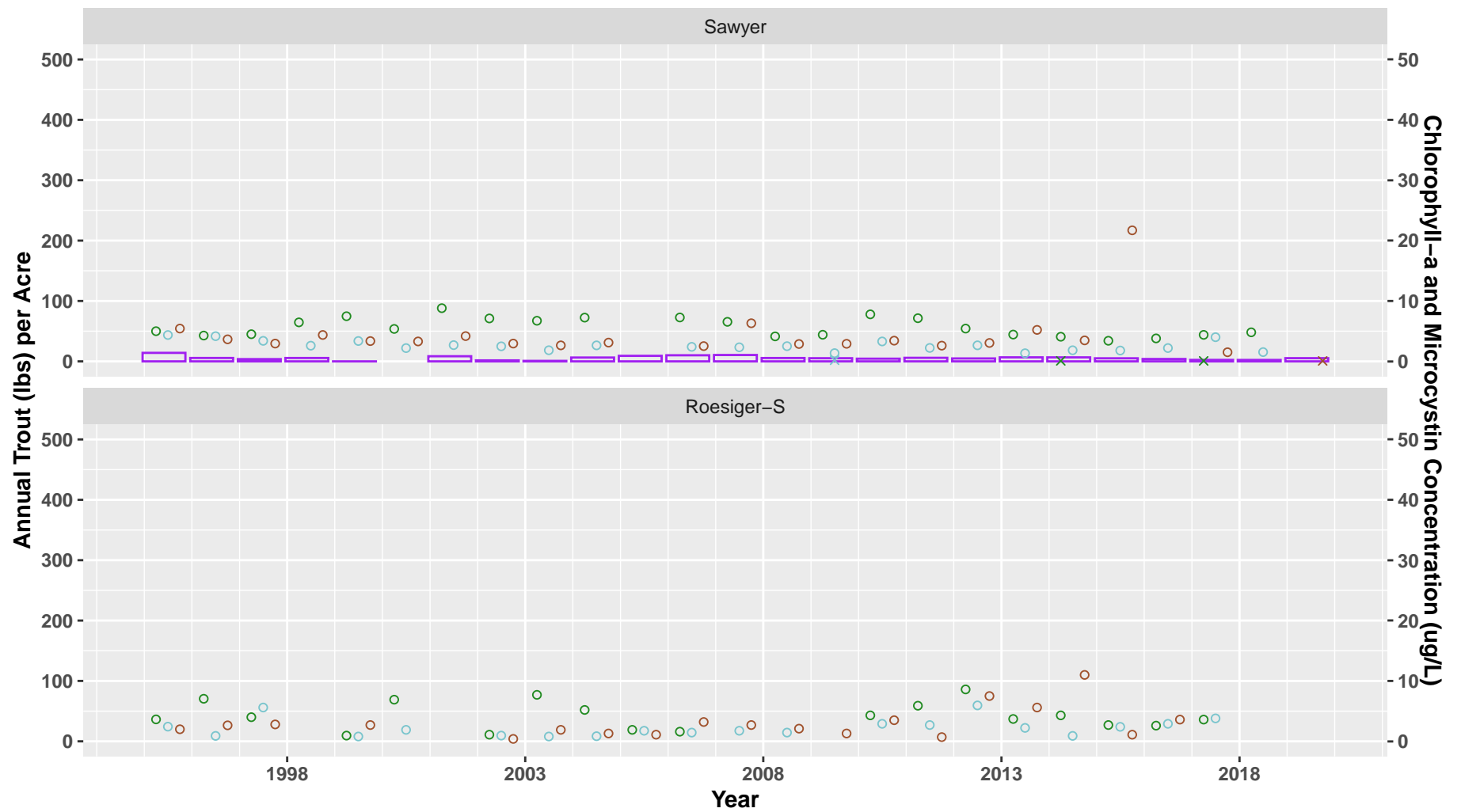


Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

Shading ● within extents

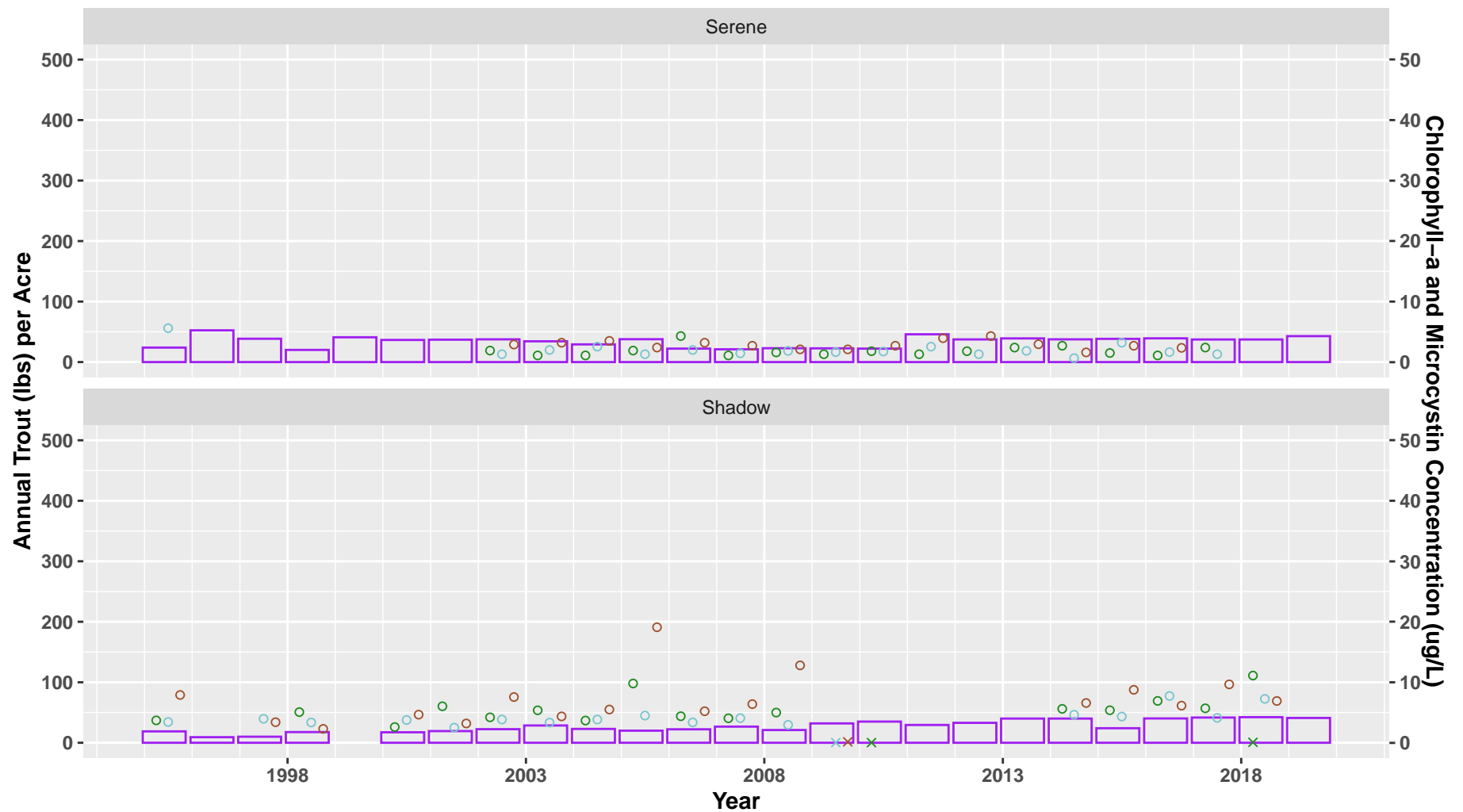




Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

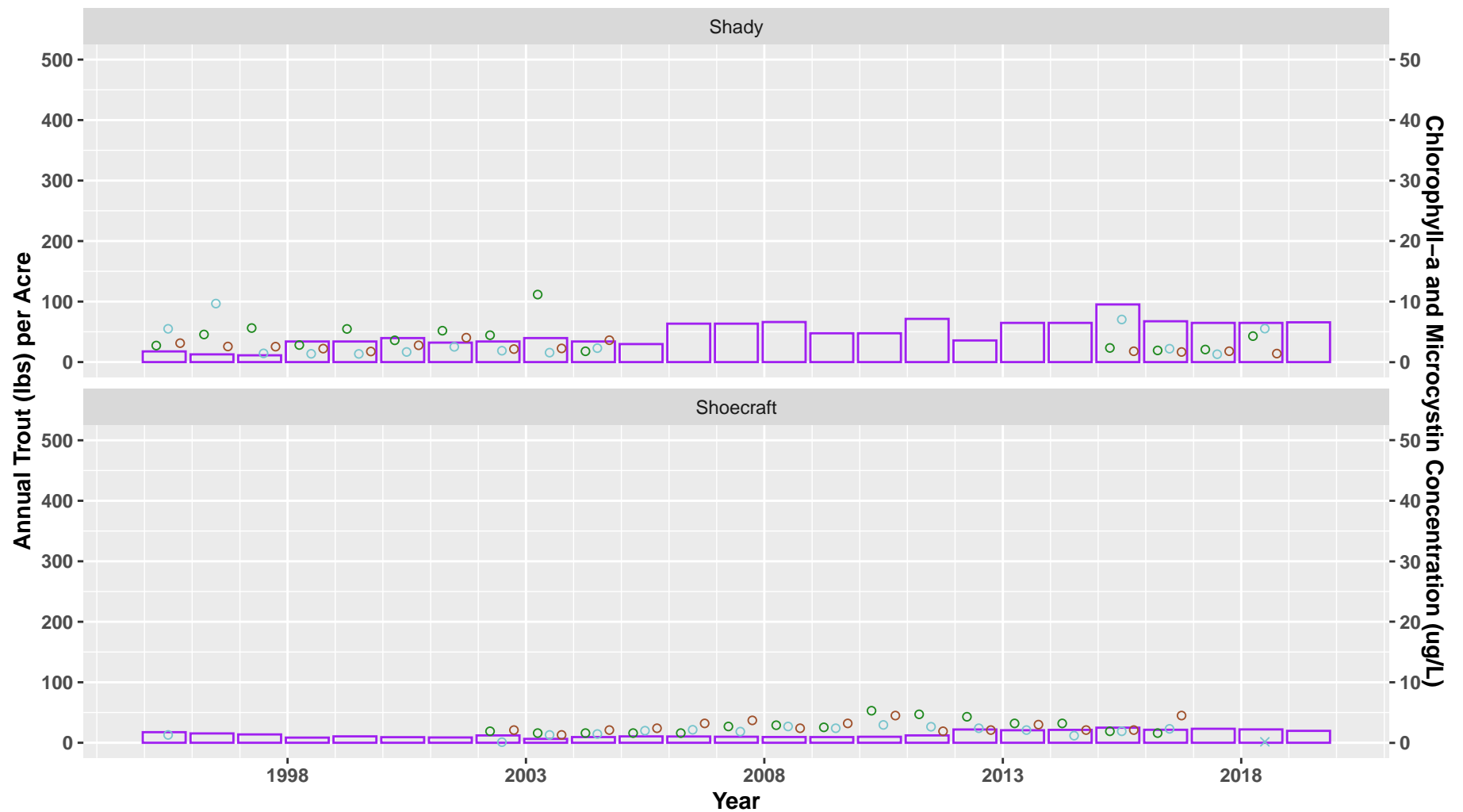
Shading ● within extents



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

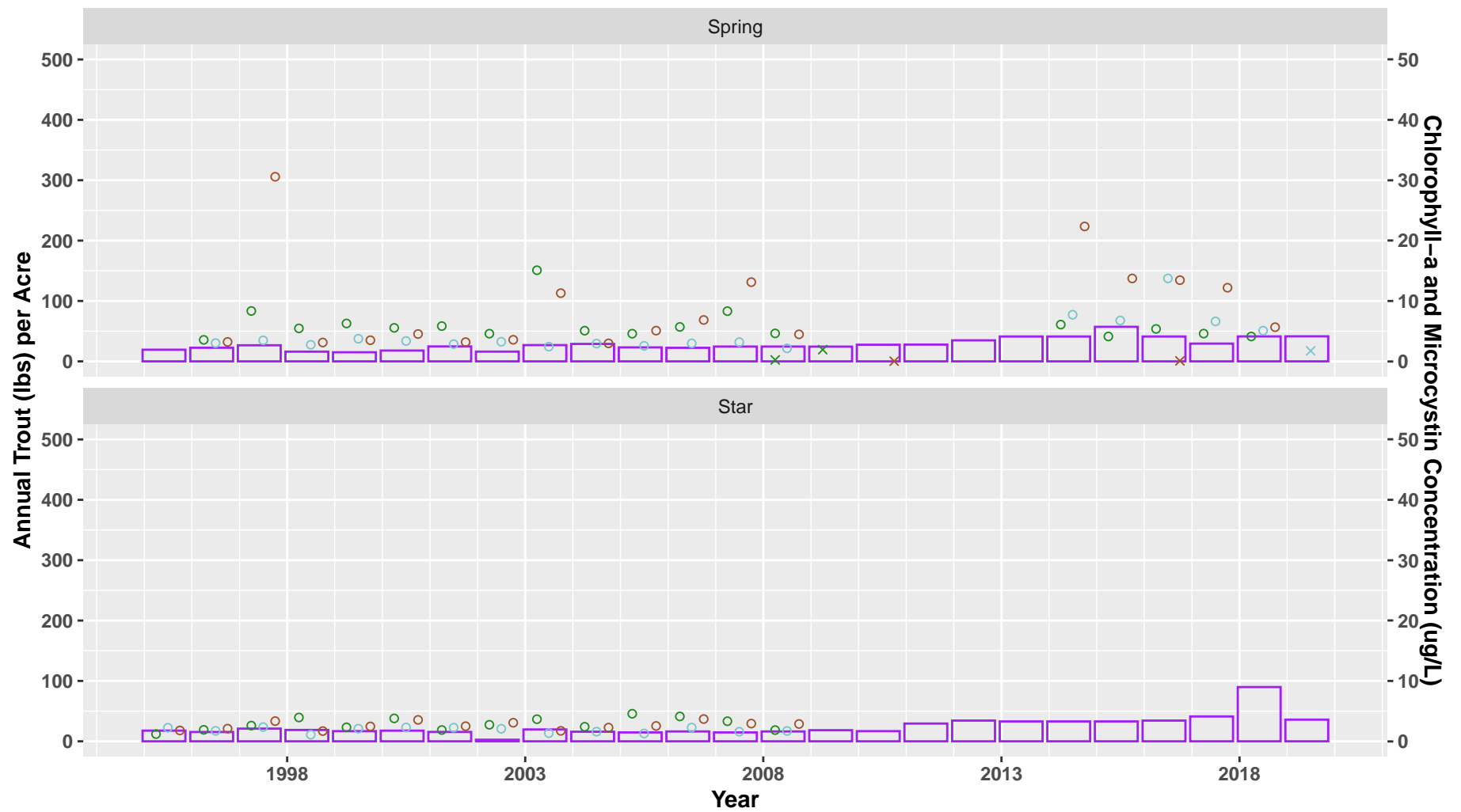
Shading ● within extents



Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

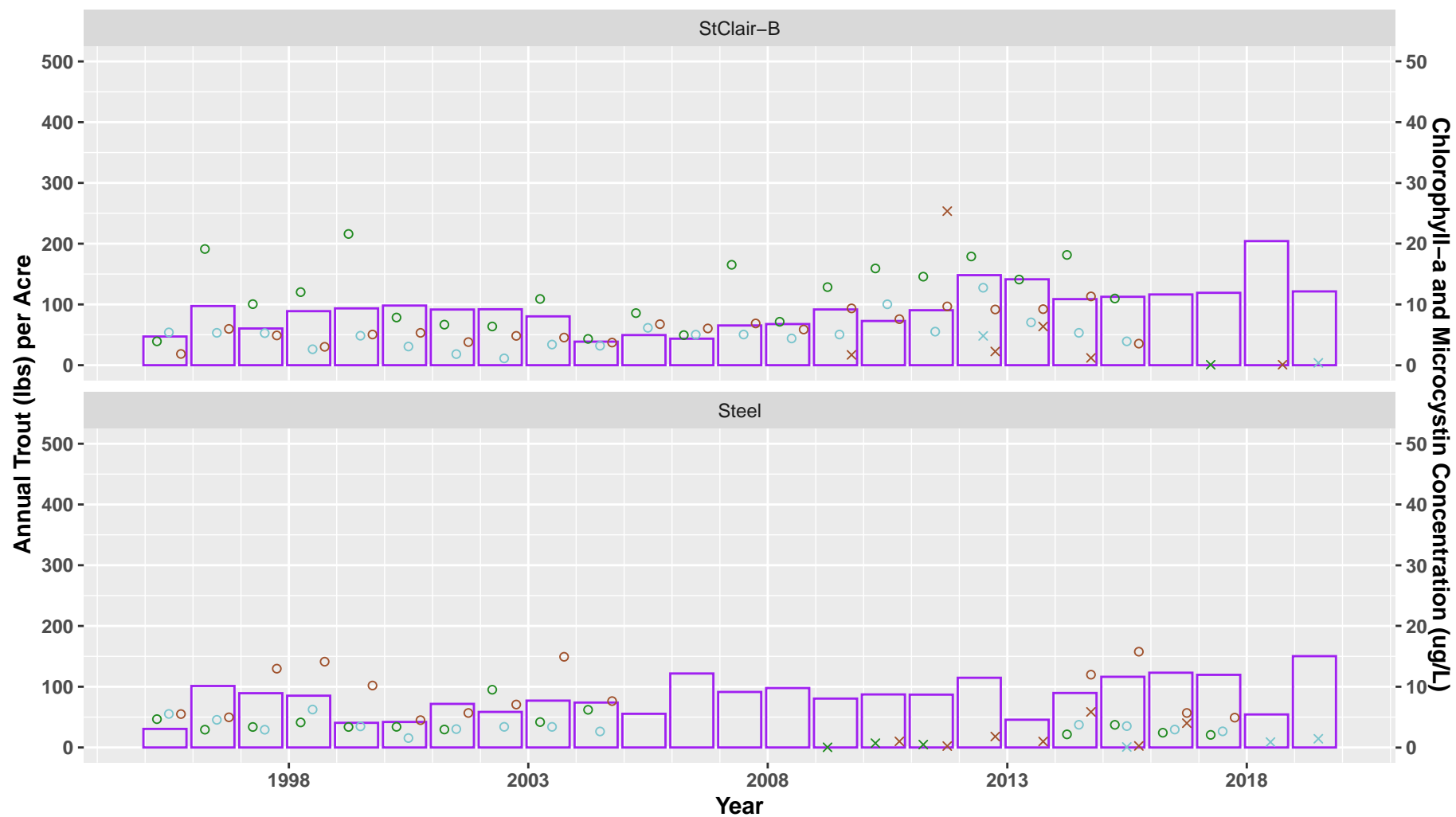
Shading ● within extents

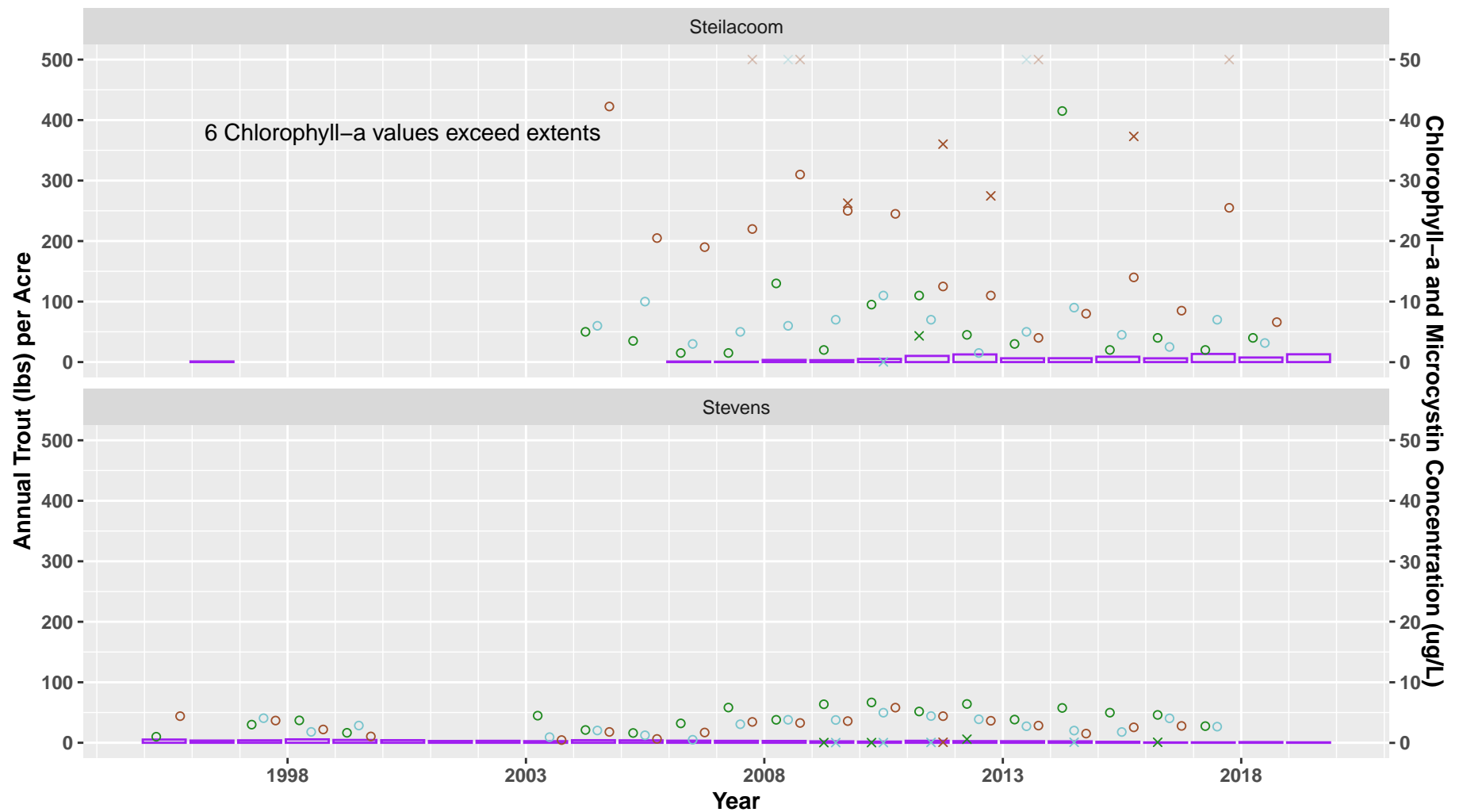


Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

Shading ● within extents

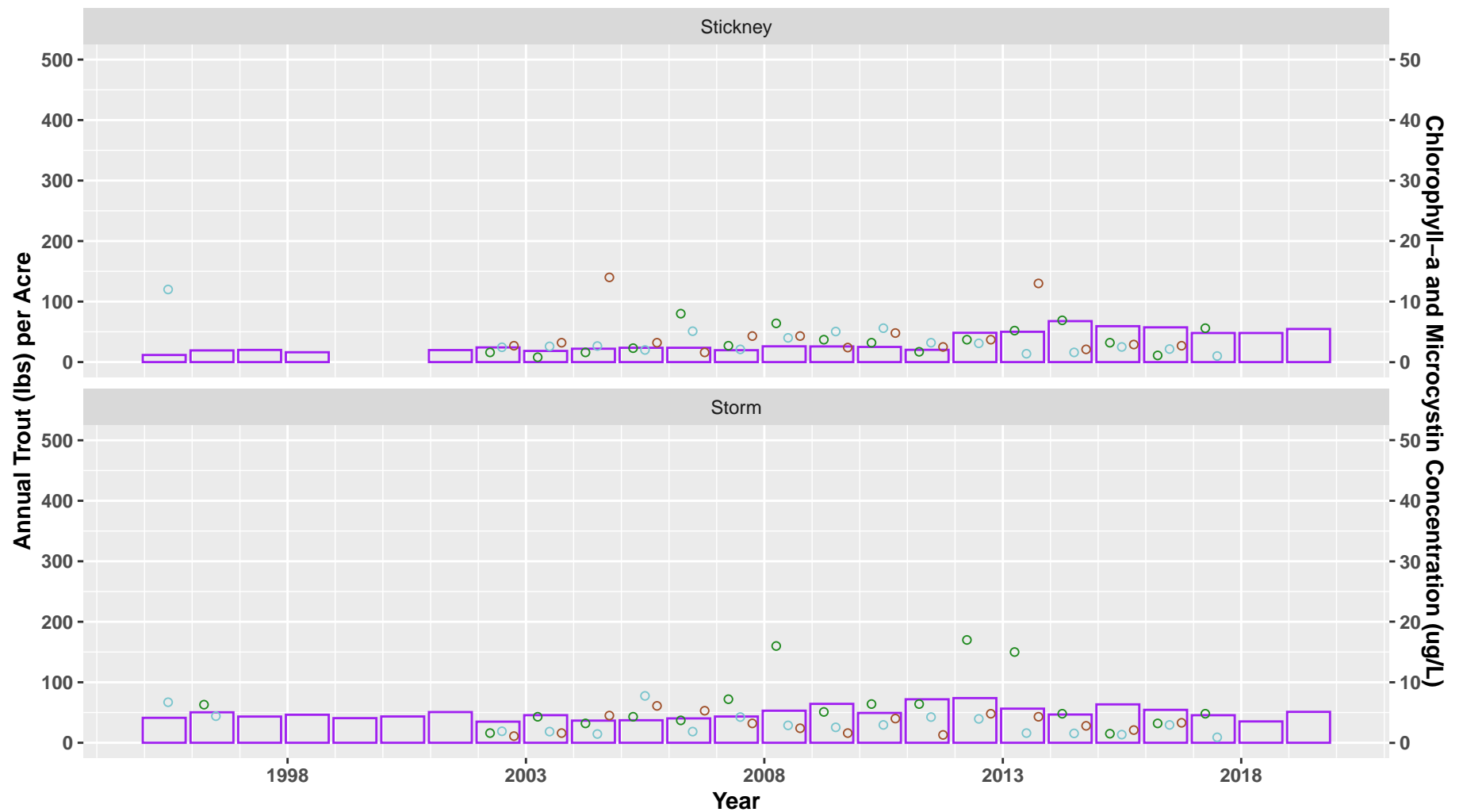


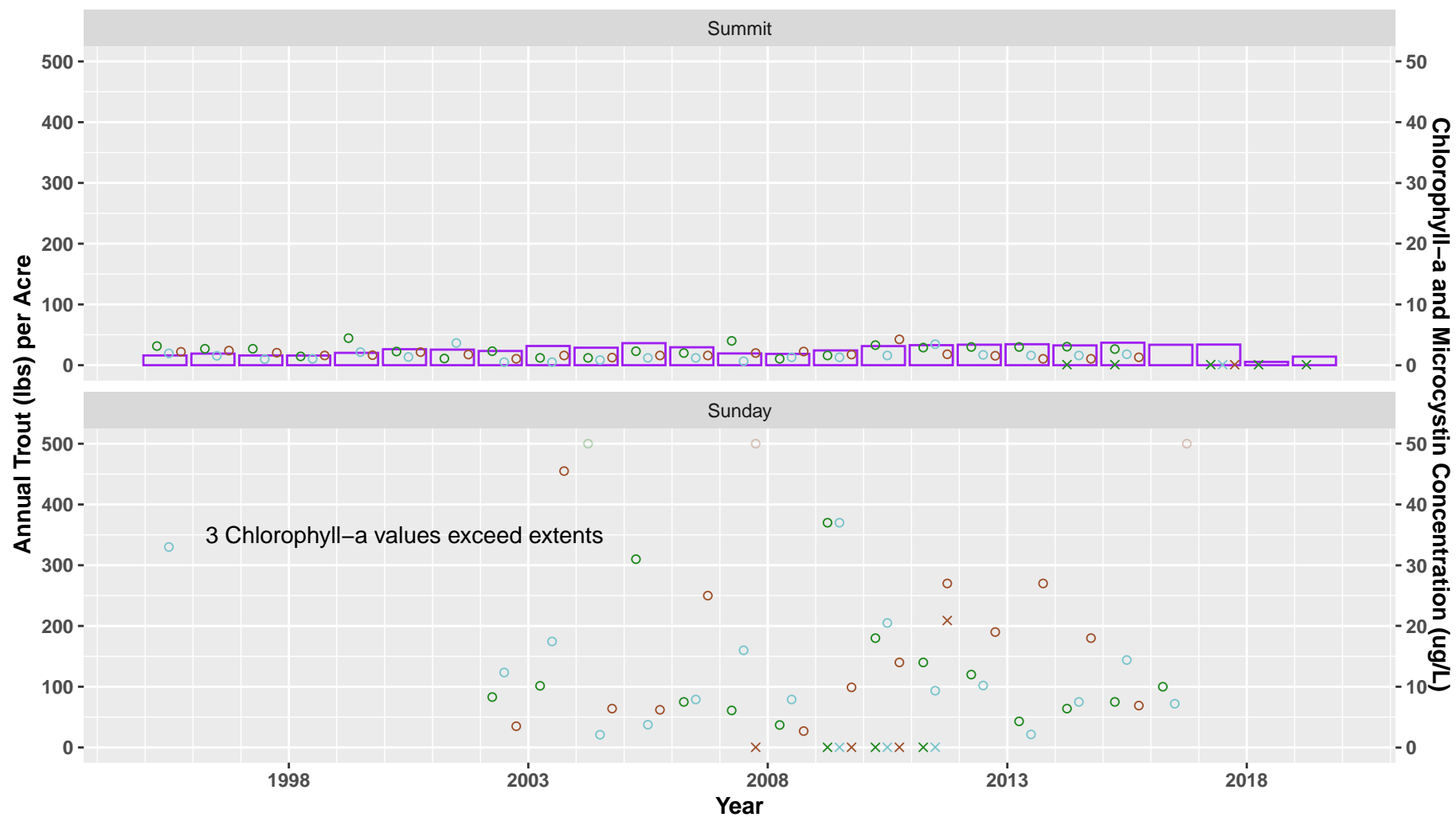


Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

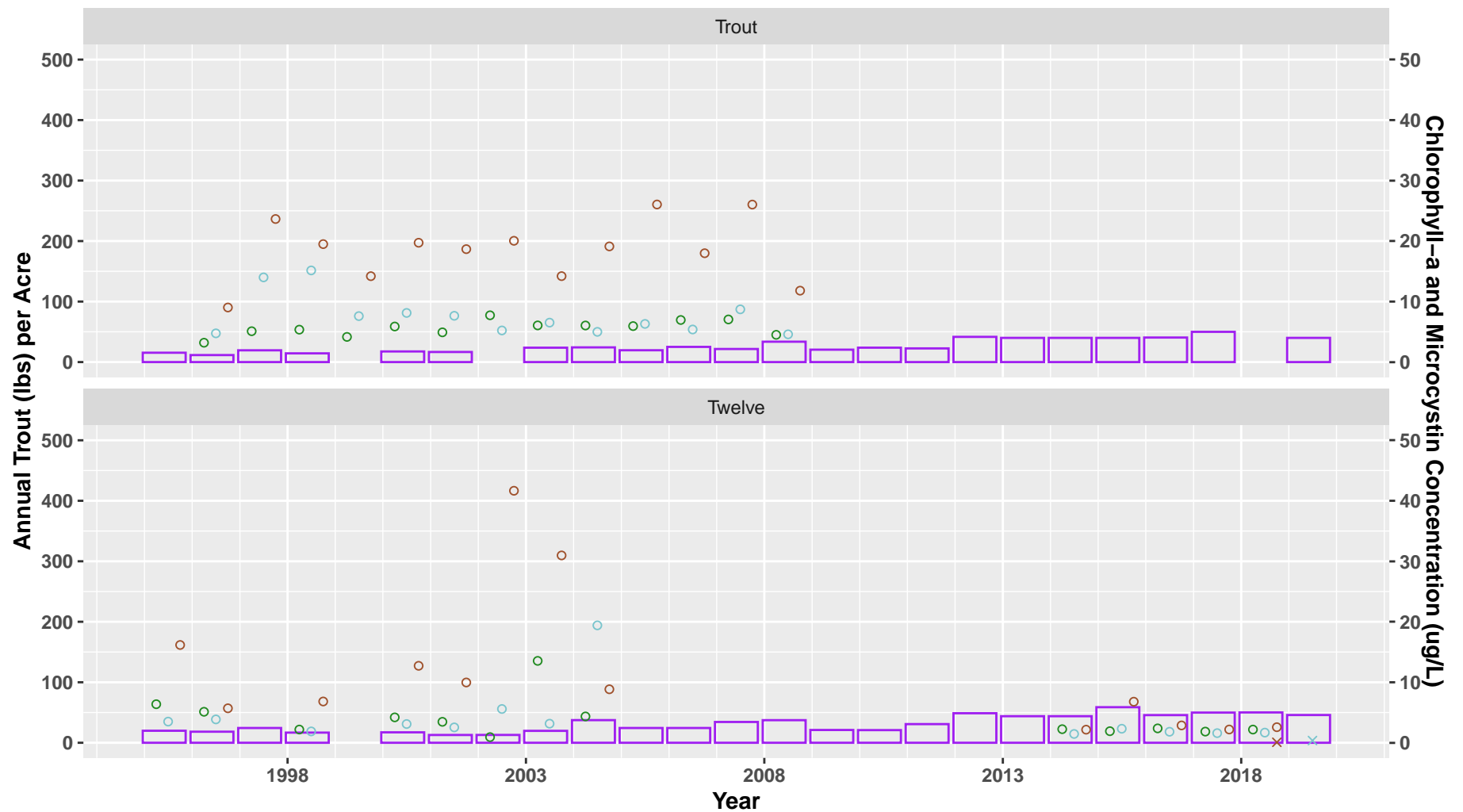


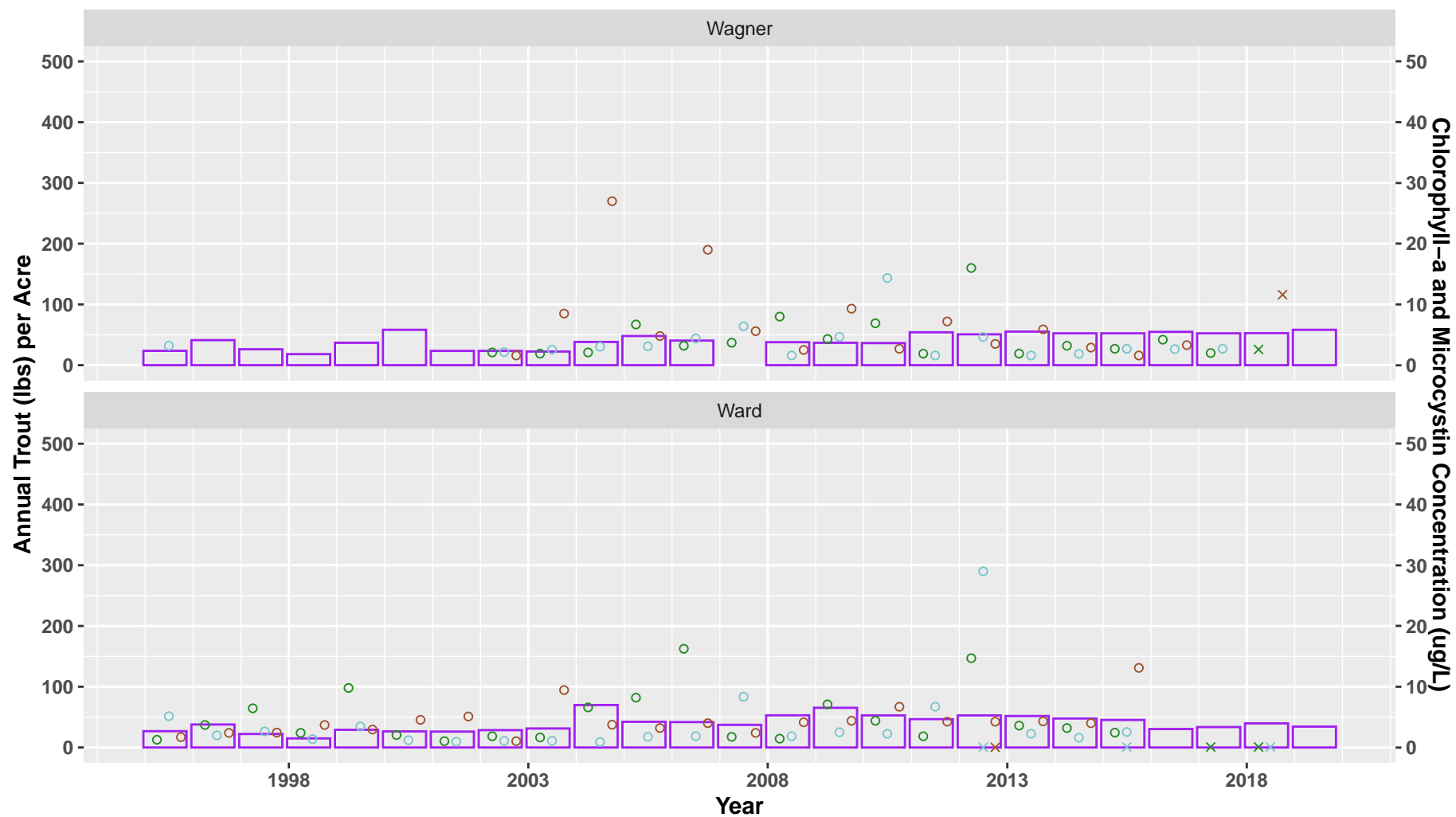


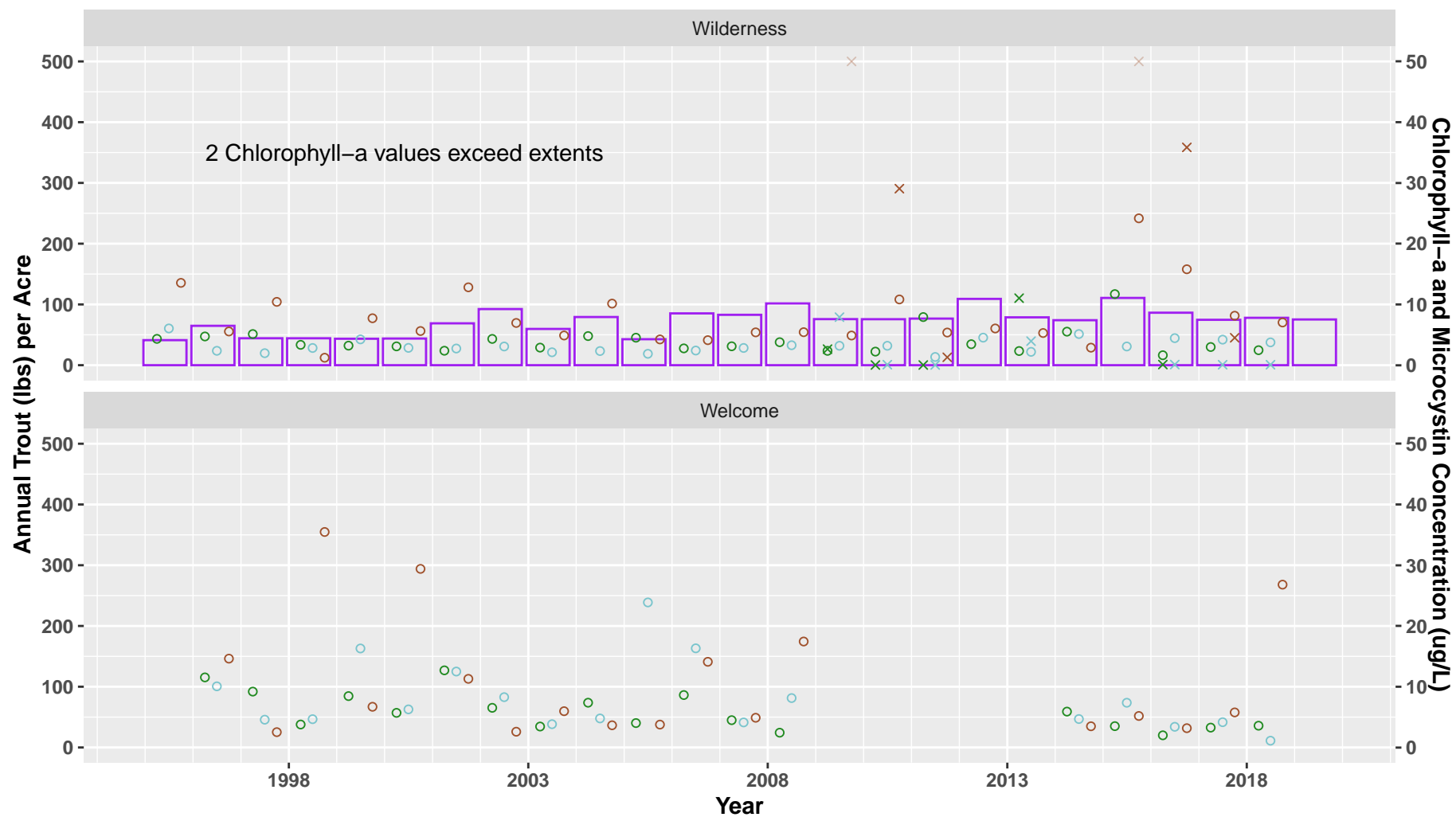
Shading ● within extents ● outside of extents

Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall





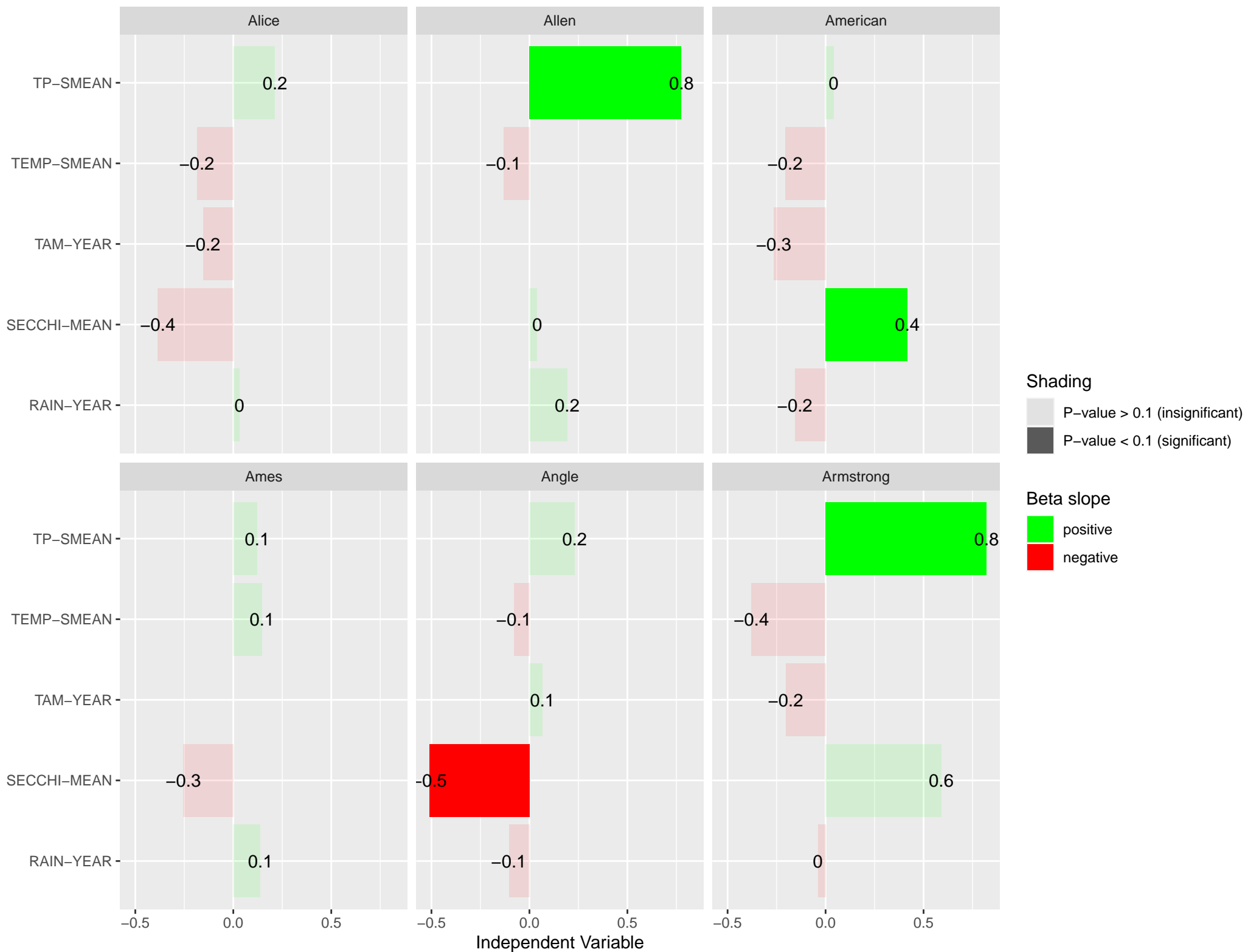


Shading ● within extents ● outside of extents

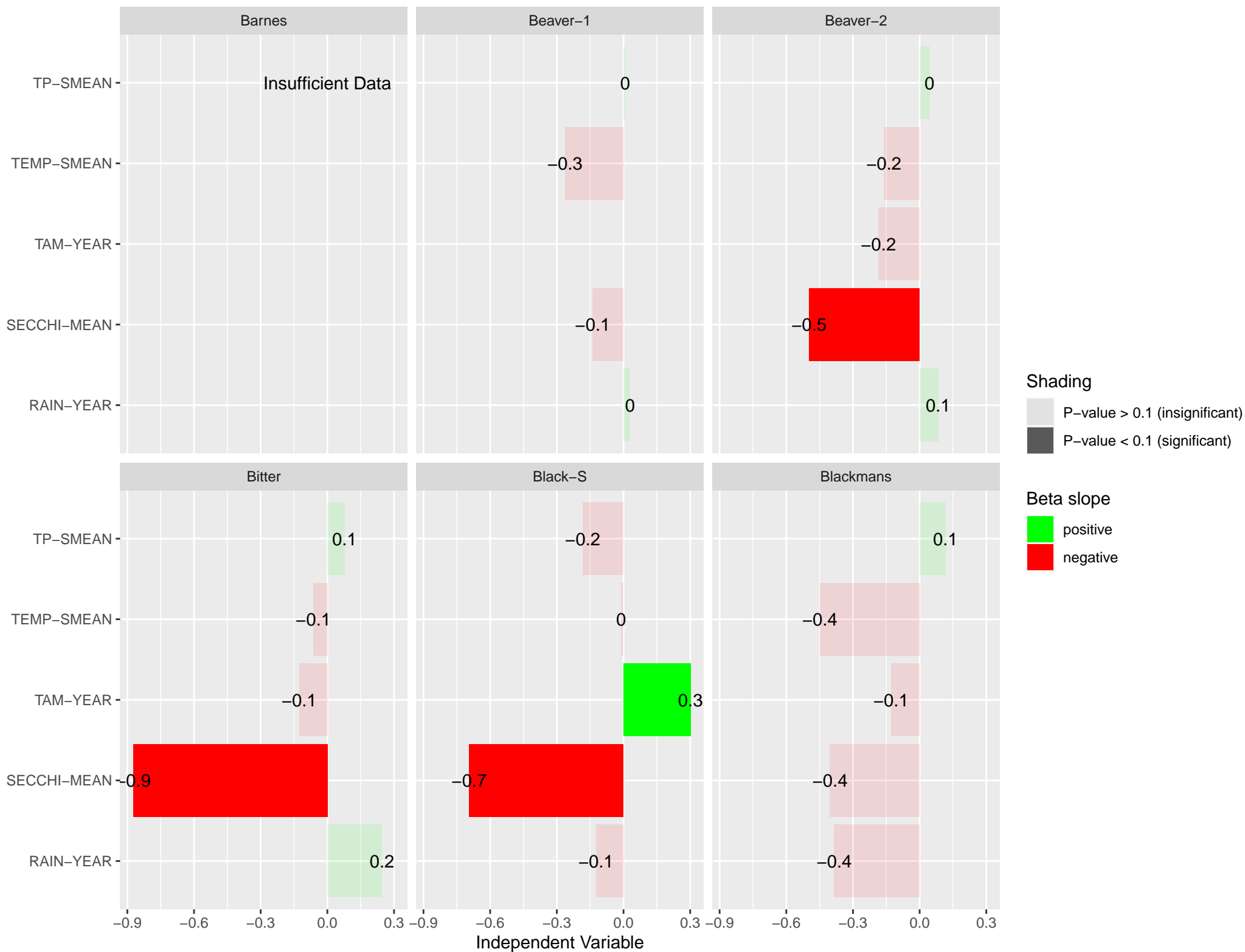
Parameter ○ Chlorophyll-a × Microcystin □ Trout lbs per Acre

Season ● Spring ● Summer ● Fall

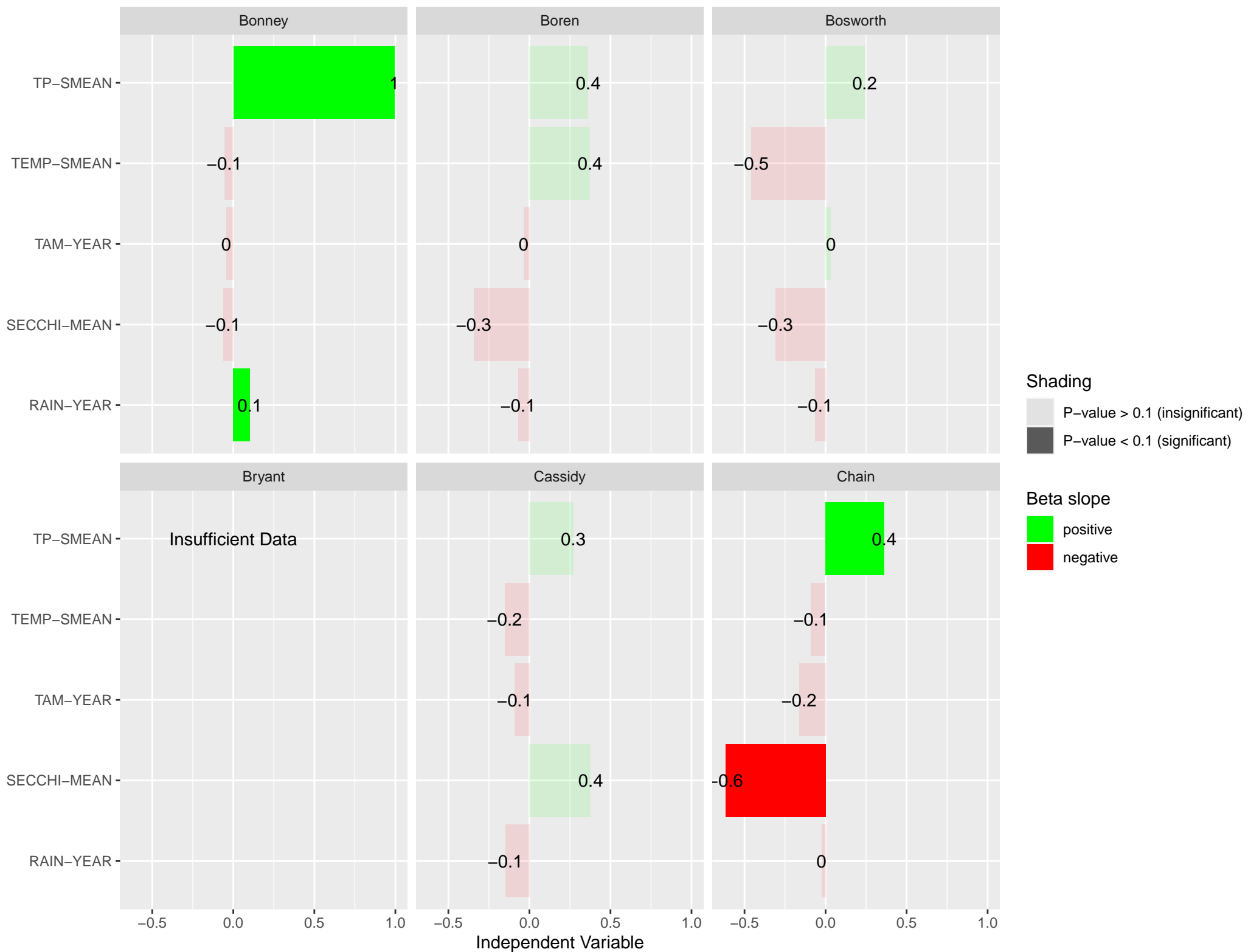
Multiple Regression Results for CHLA-SMEAN (Annual data)



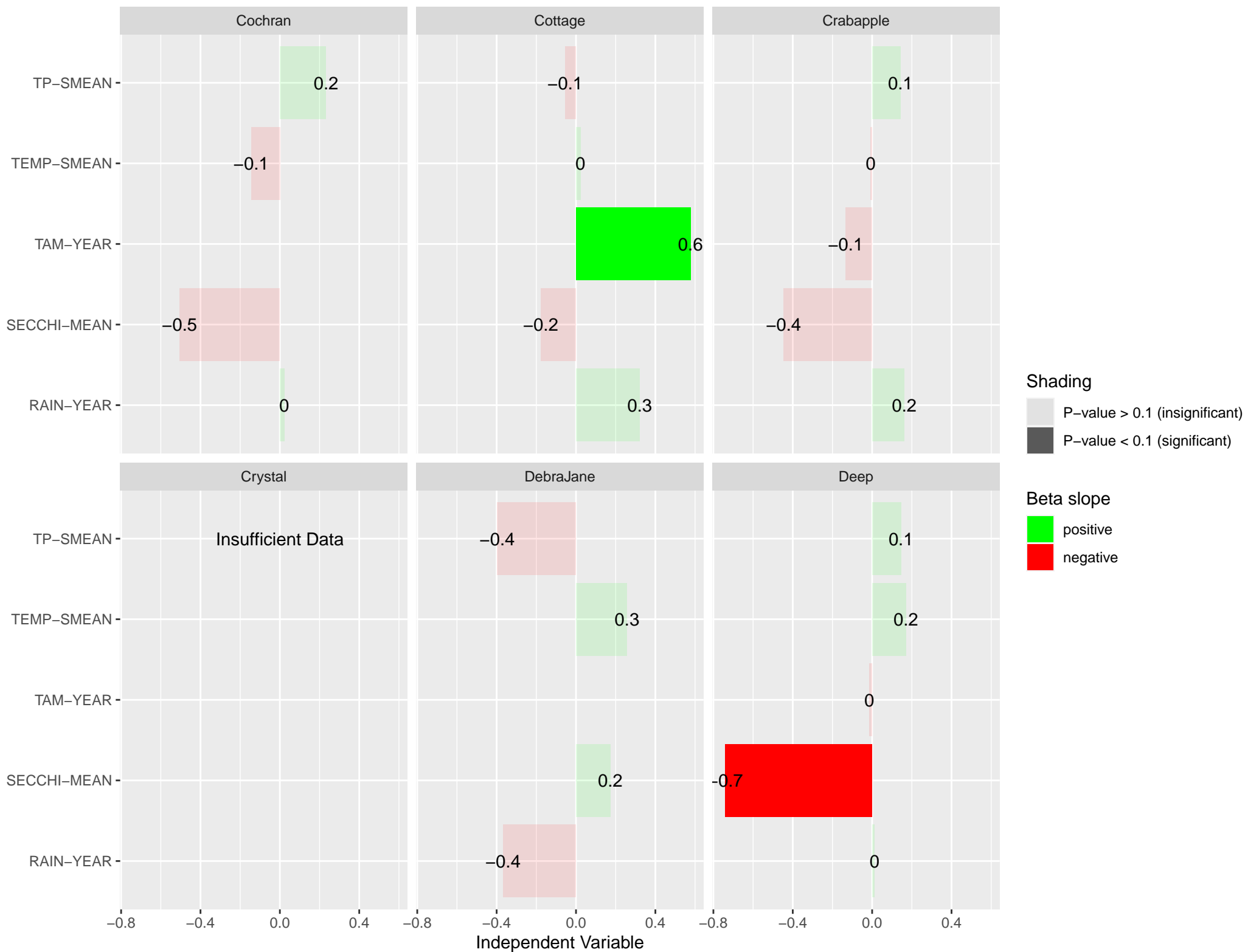
Multiple Regression Results for CHLA-SMEAN (Annual data)



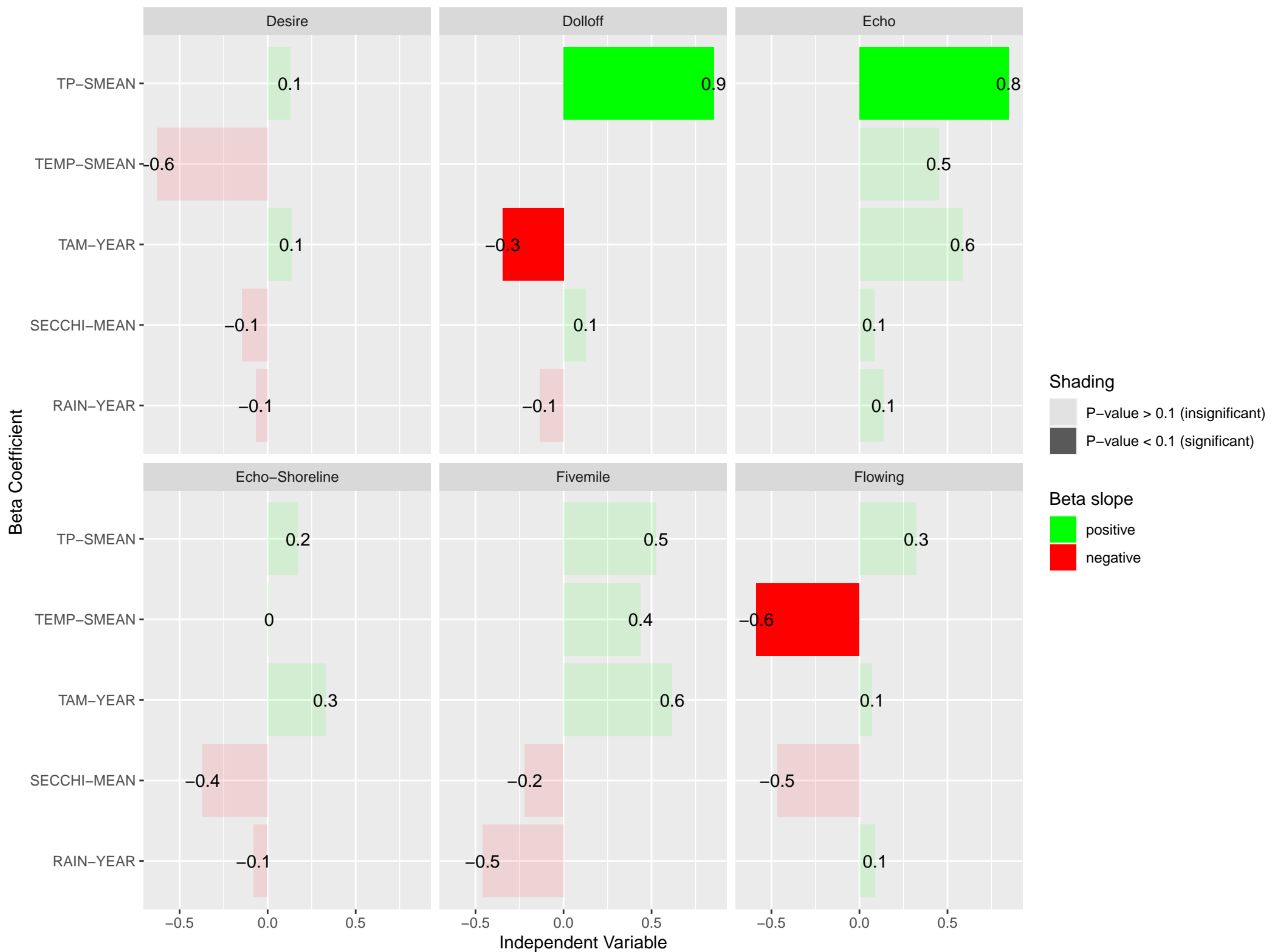
Multiple Regression Results for CHLA-SMEAN (Annual data)



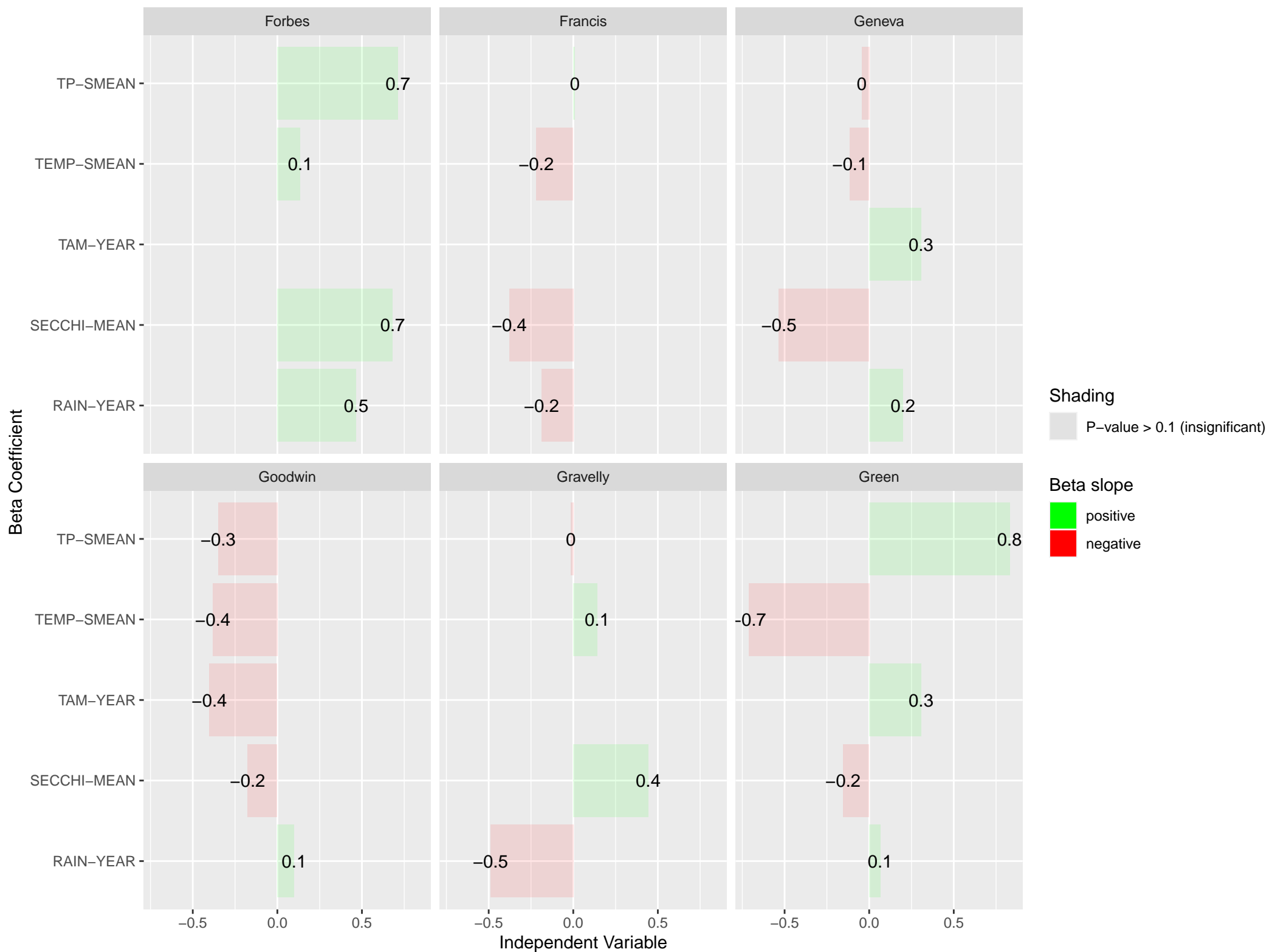
Multiple Regression Results for CHLA-SMEAN (Annual data)



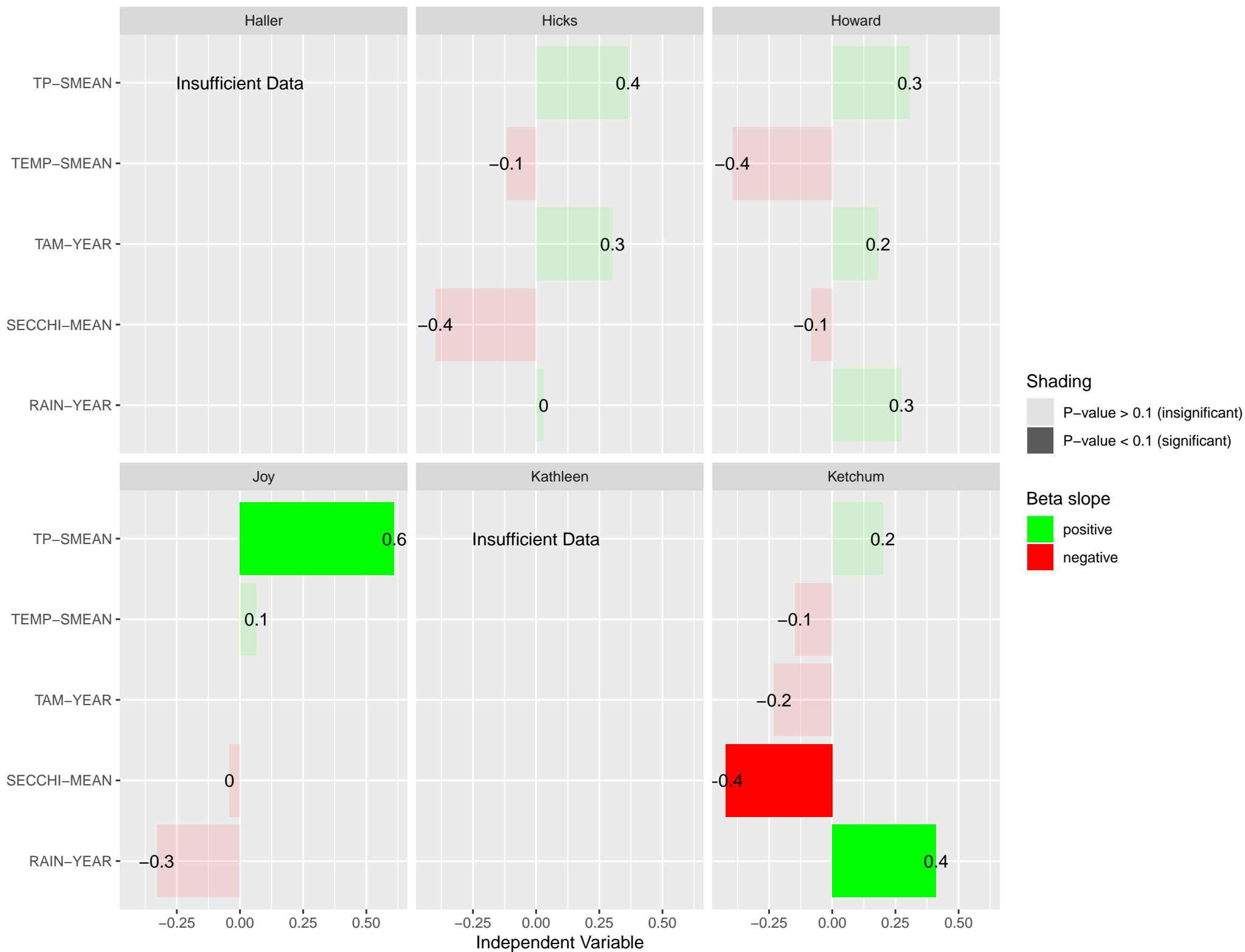
Multiple Regression Results for CHLA-SMEAN (Annual data)



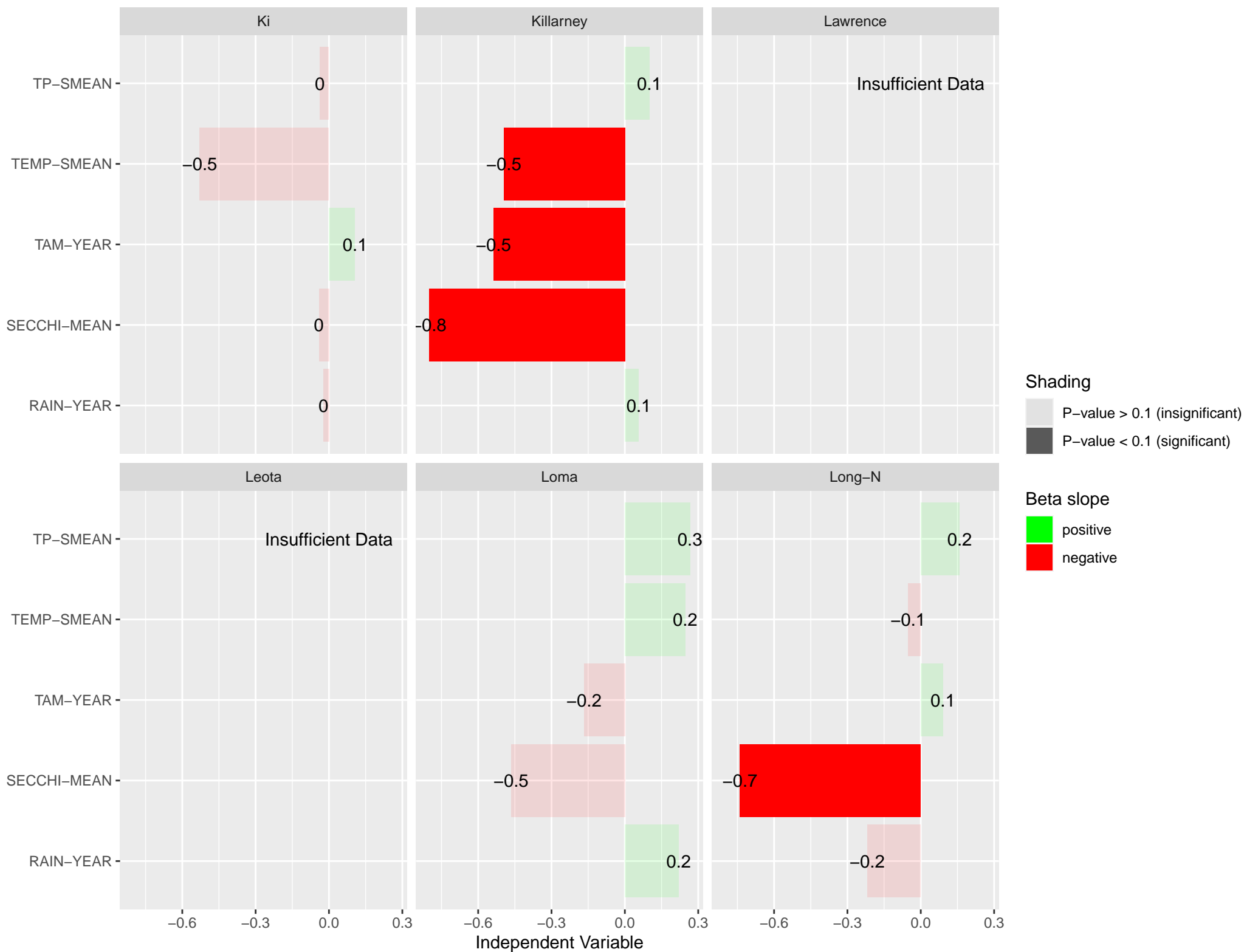
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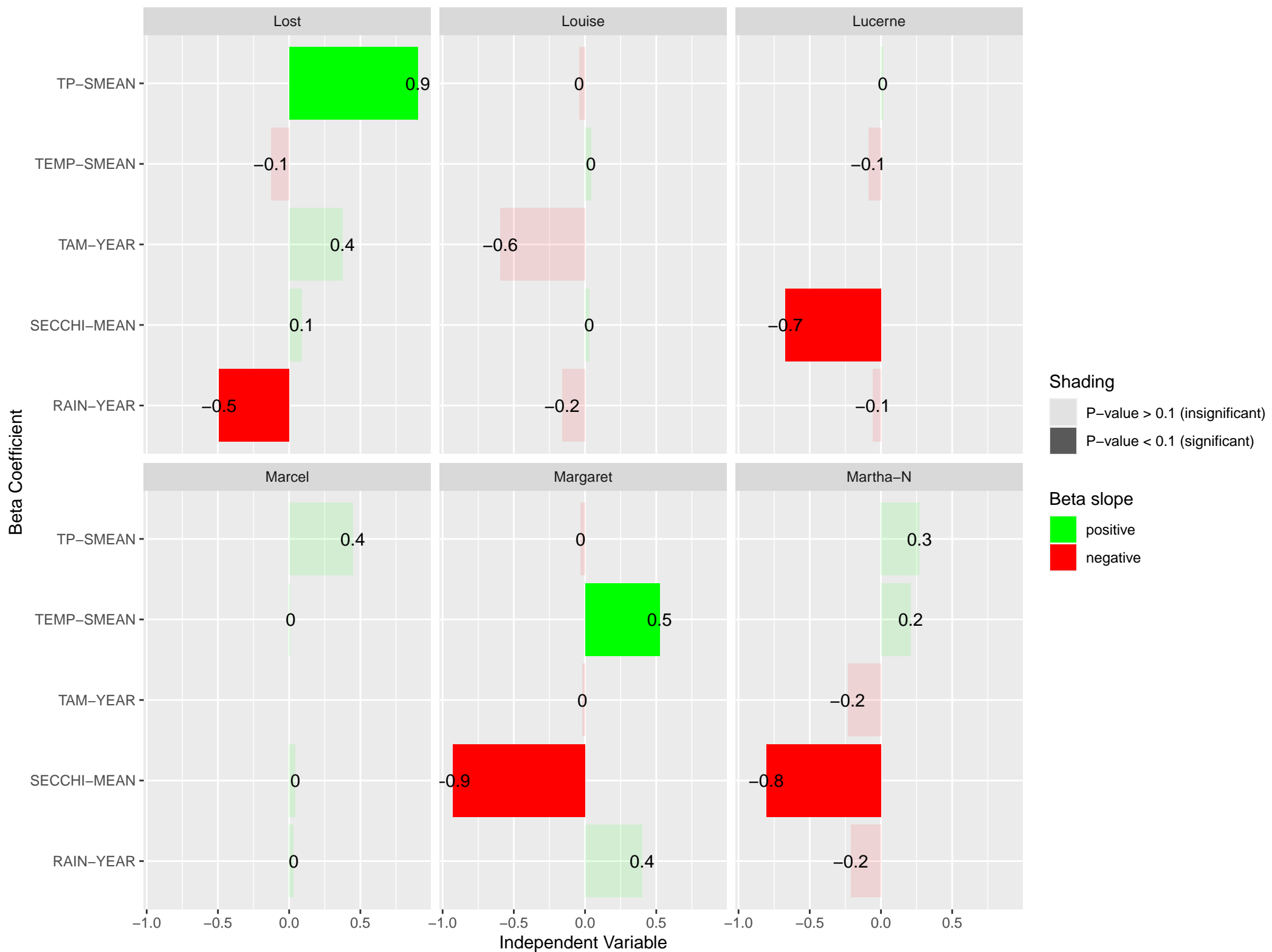
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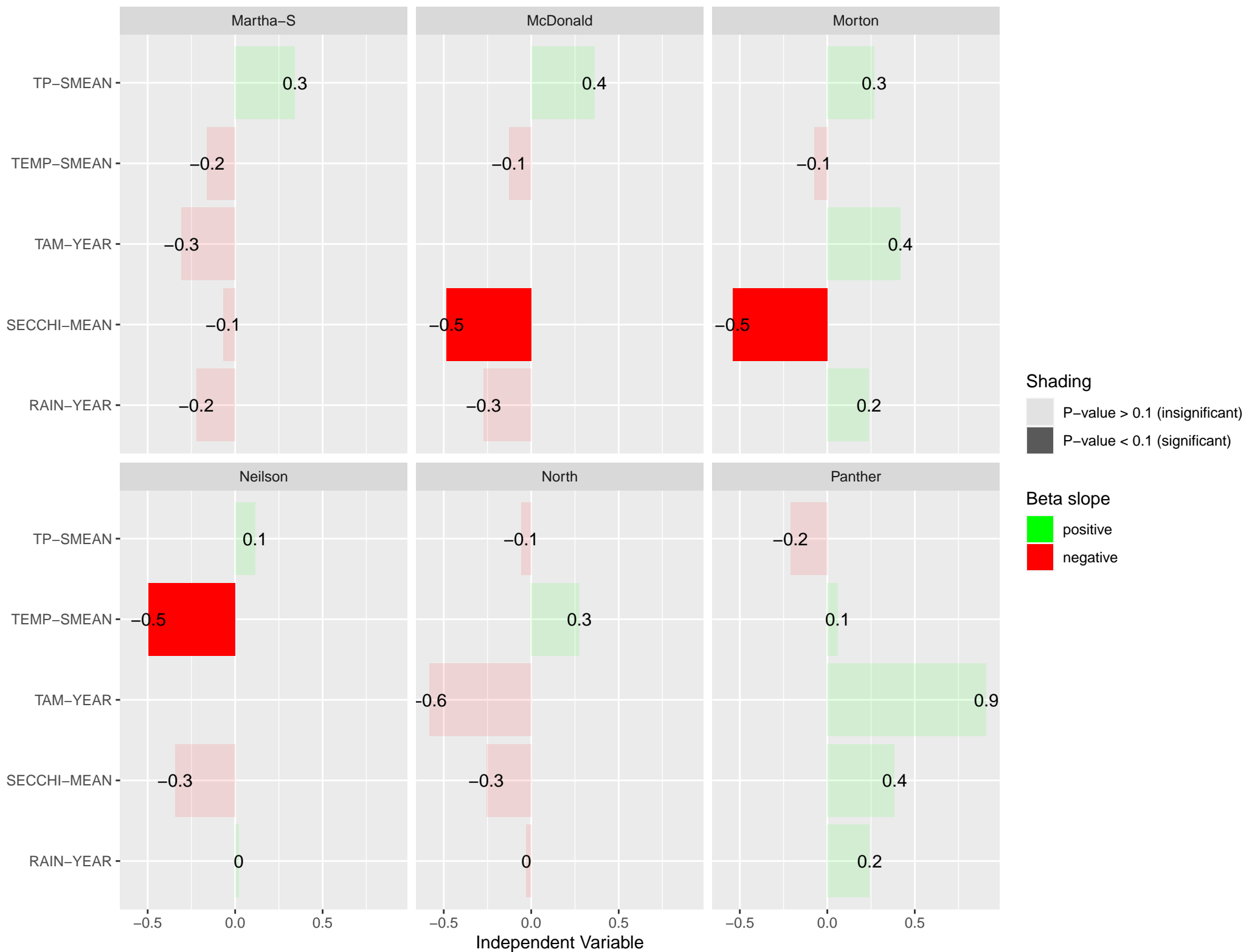
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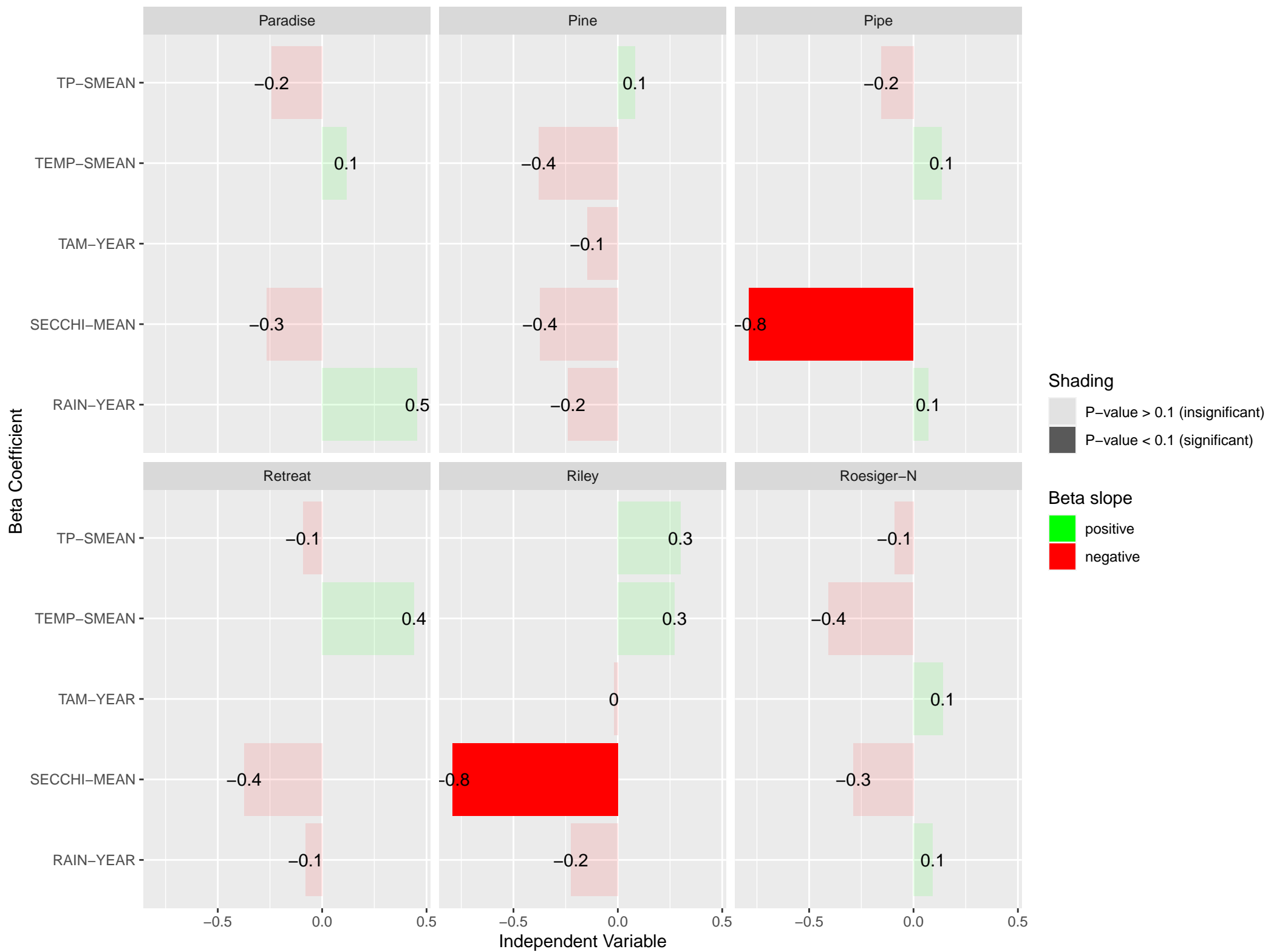
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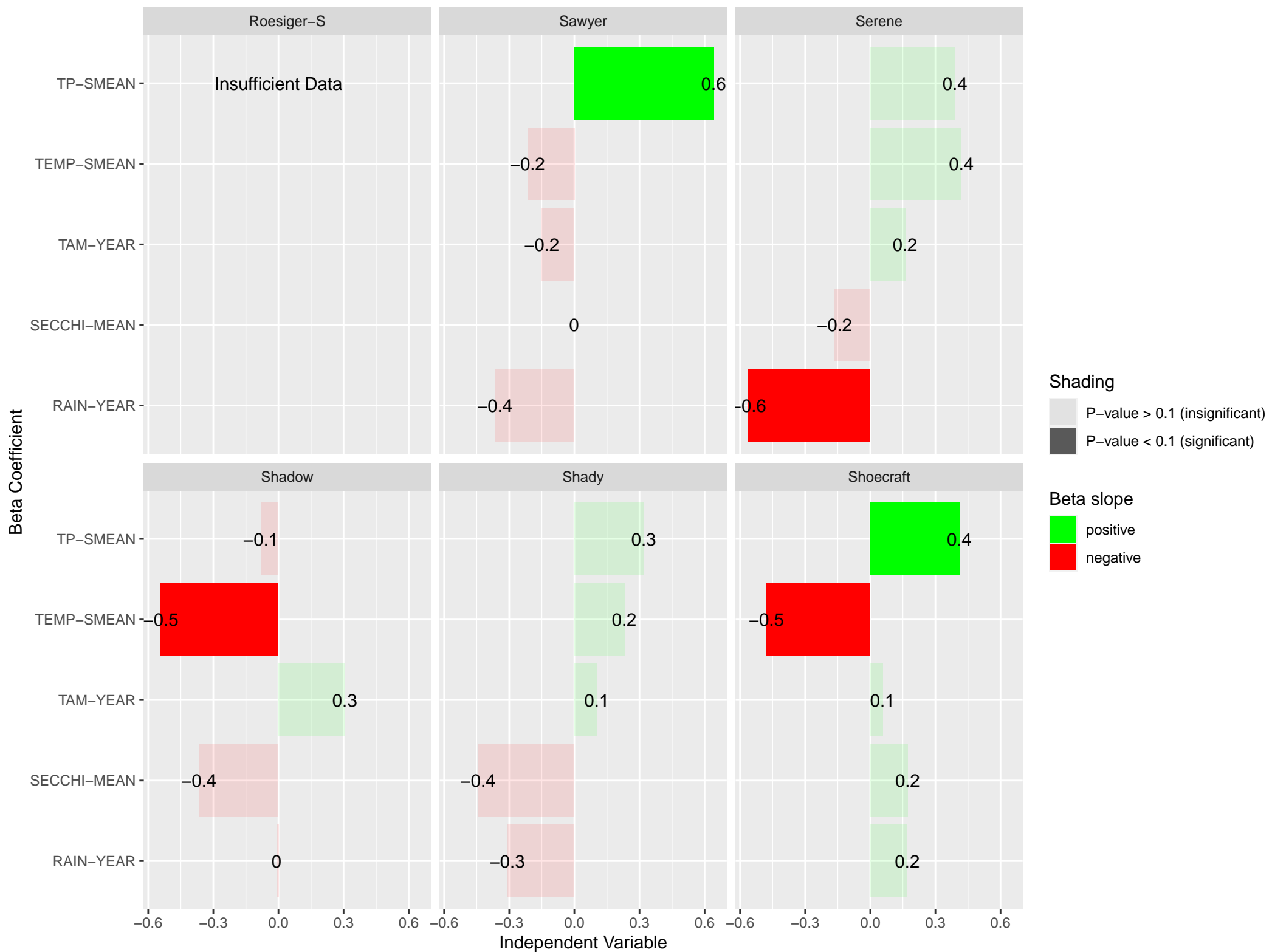
Multiple Regression Results for CHLA-SMEAN (Annual data)



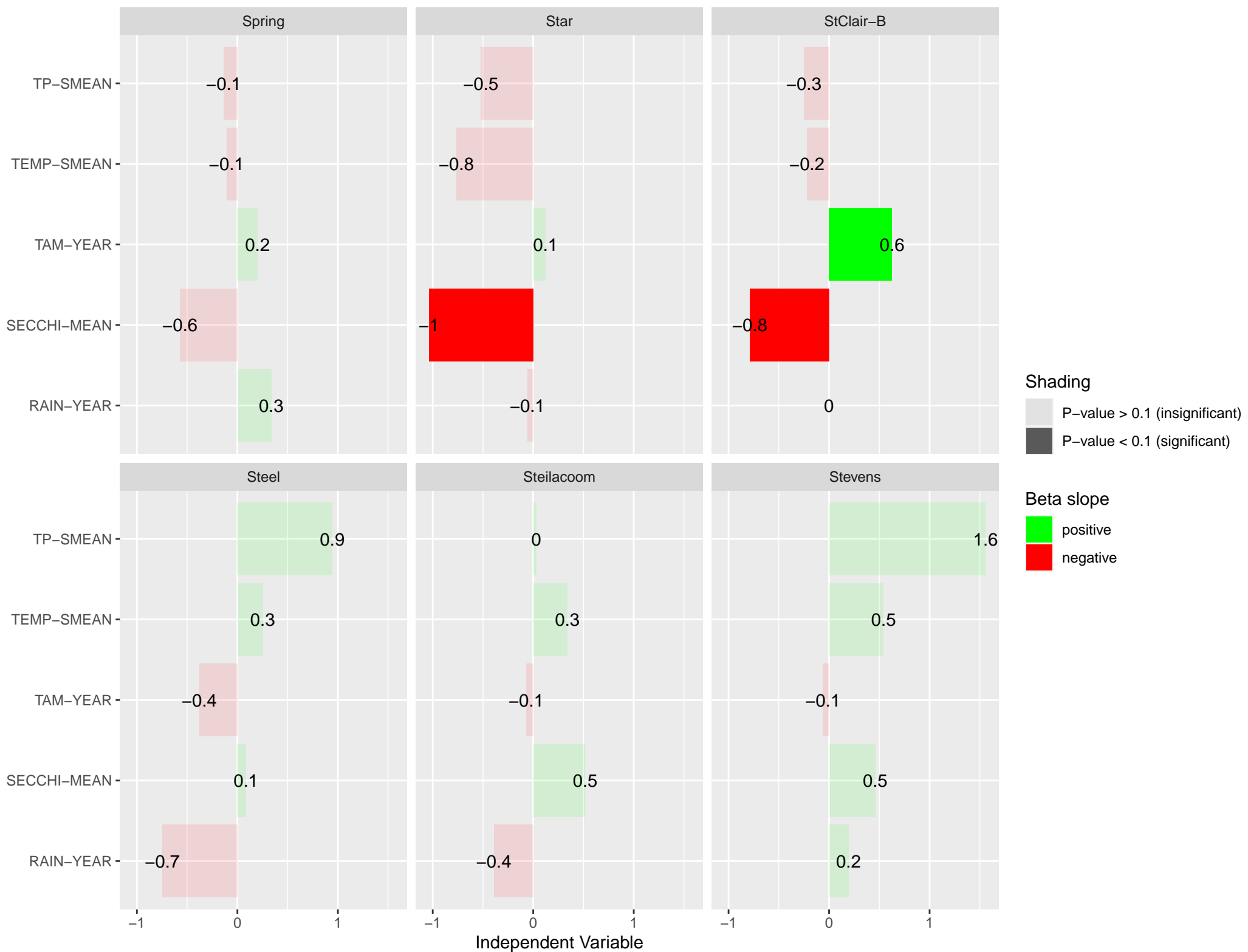
Multiple Regression Results for CHLA-SMEAN (Annual data)



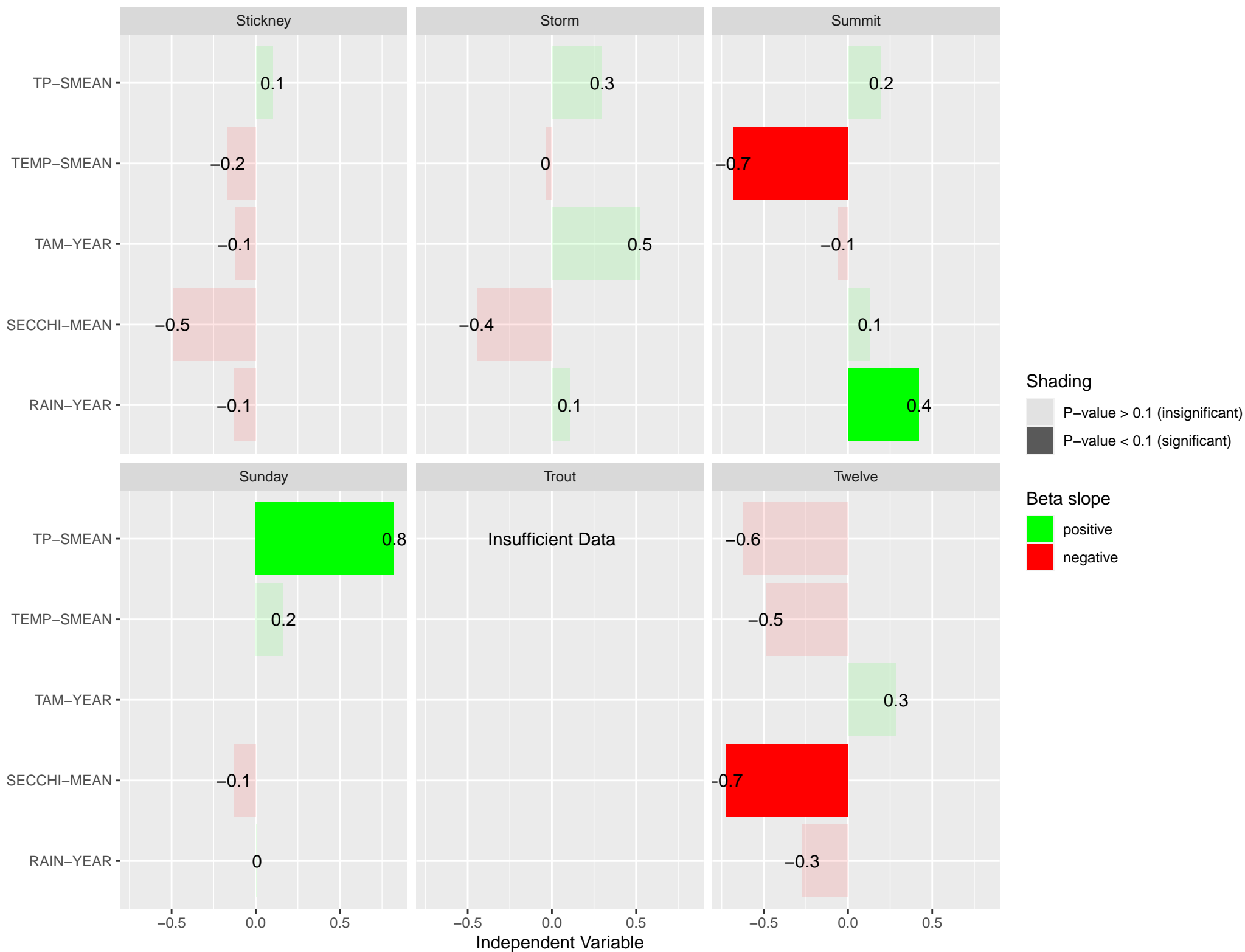
Multiple Regression Results for CHLA-SMEAN (Annual data)



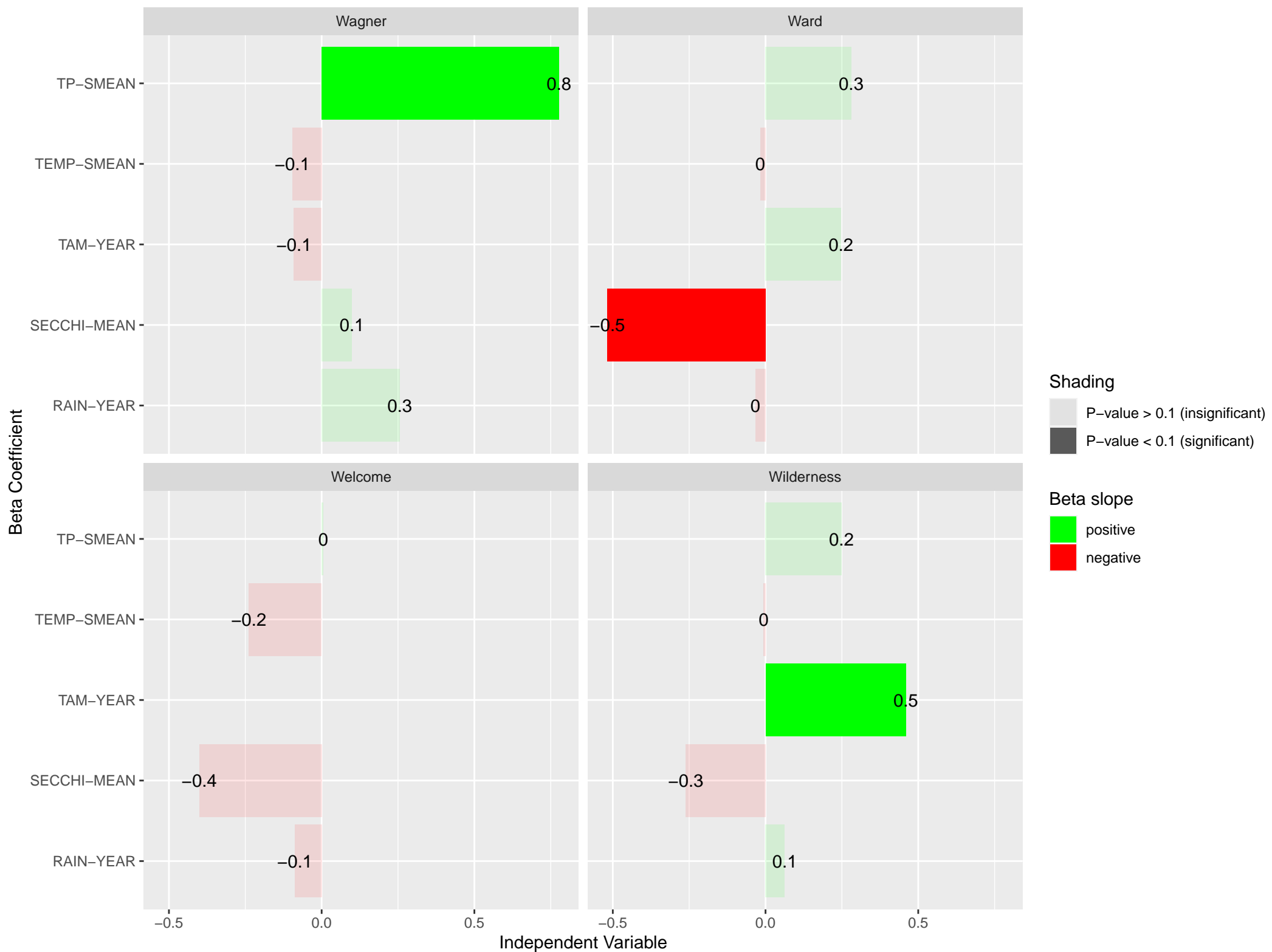
Multiple Regression Results for CHLA-SMEAN (Annual data)



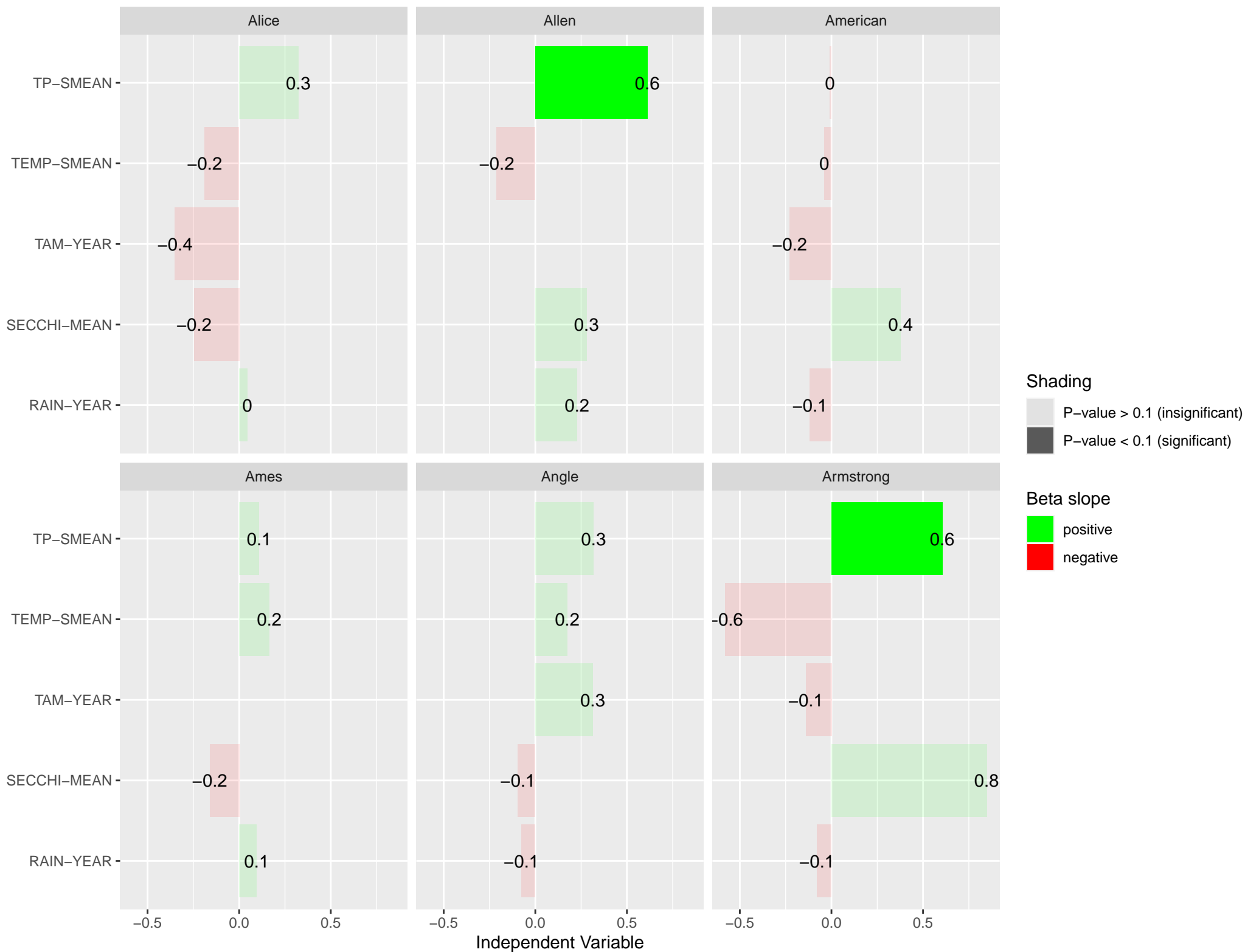
Multiple Regression Results for CHLA-SMEAN (Annual data)



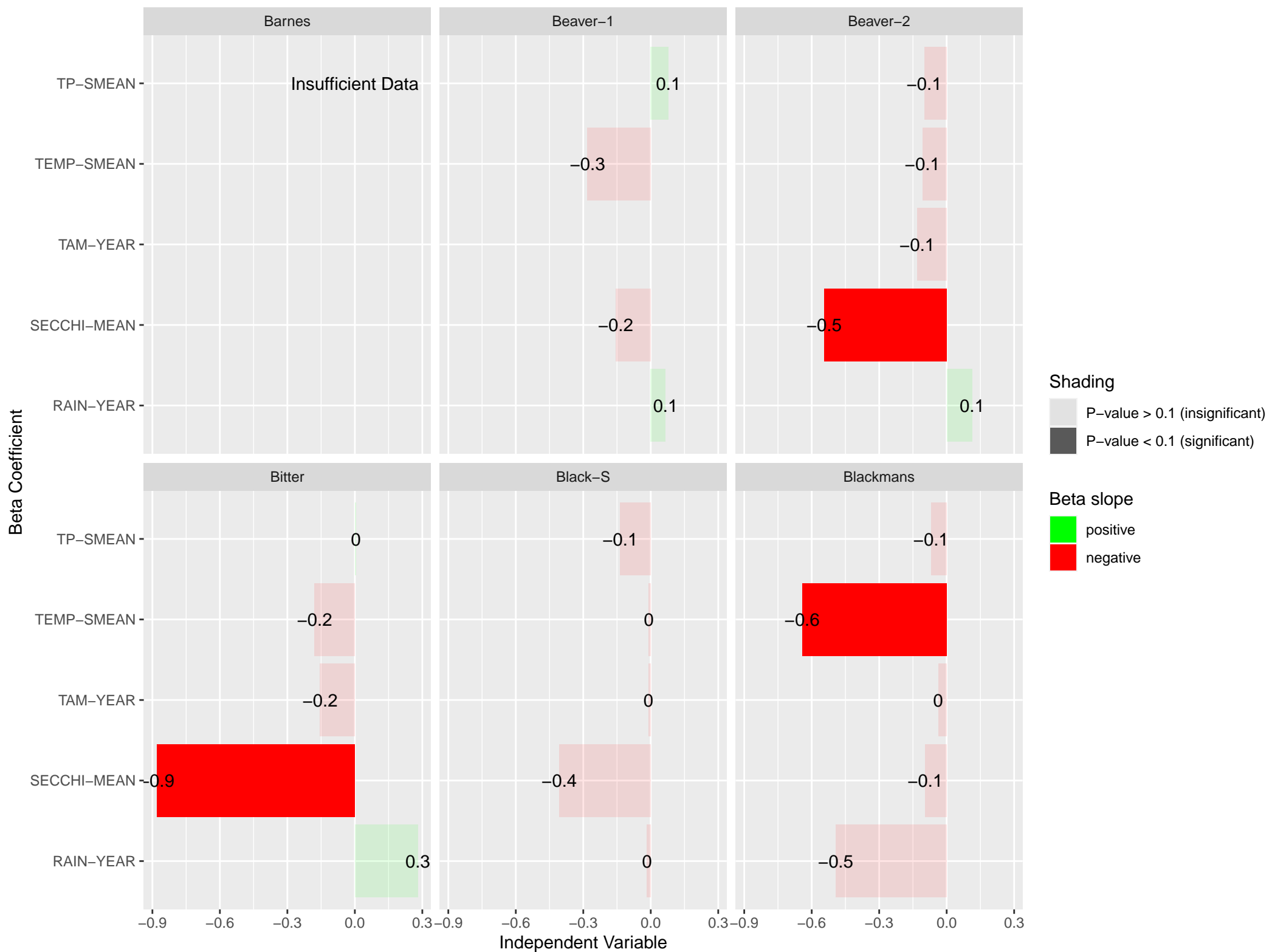
Multiple Regression Results for CHLA-SMEAN (Annual data)



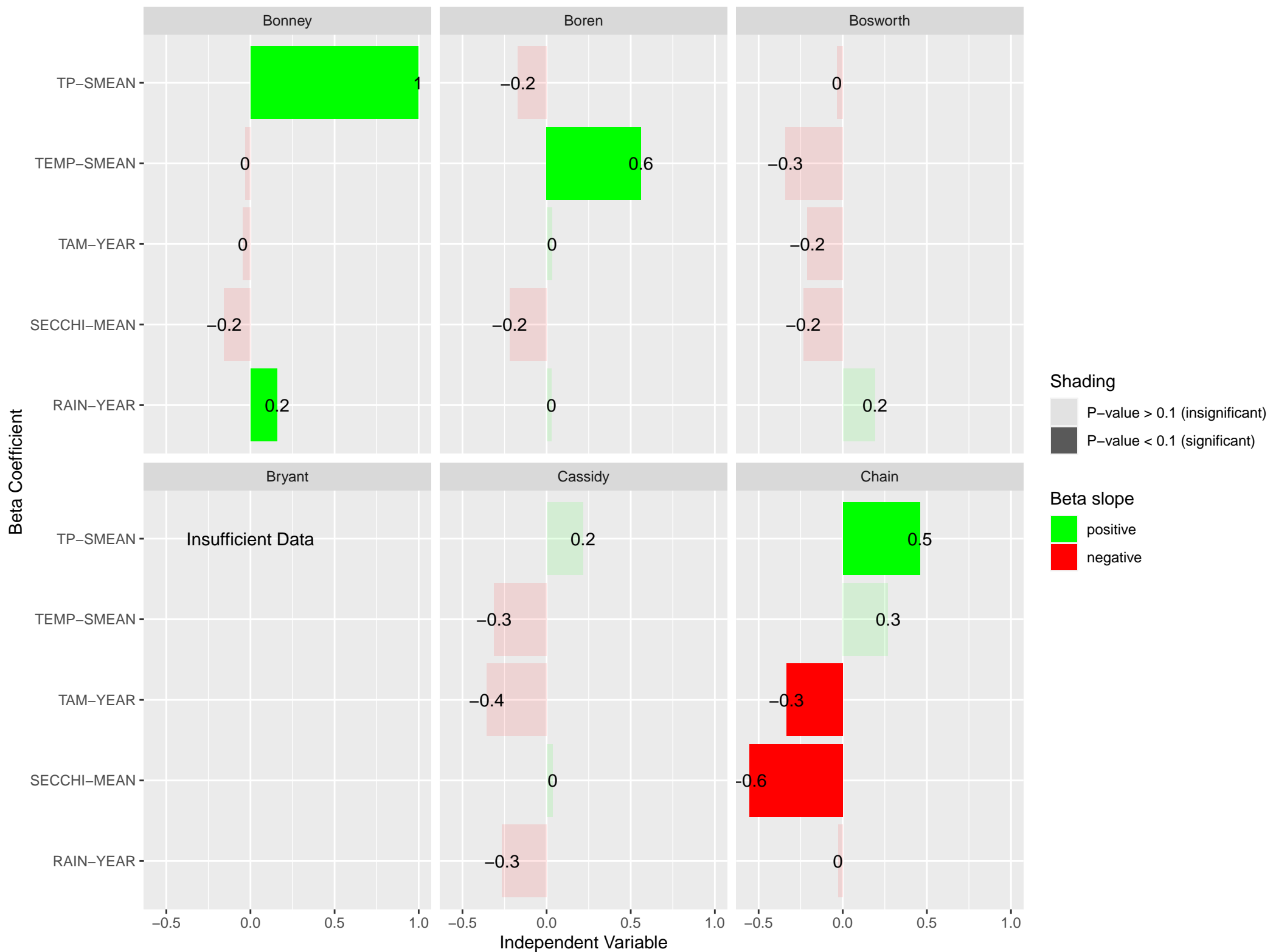
Multiple Regression Results for CHLA-SMAX (Annual data)



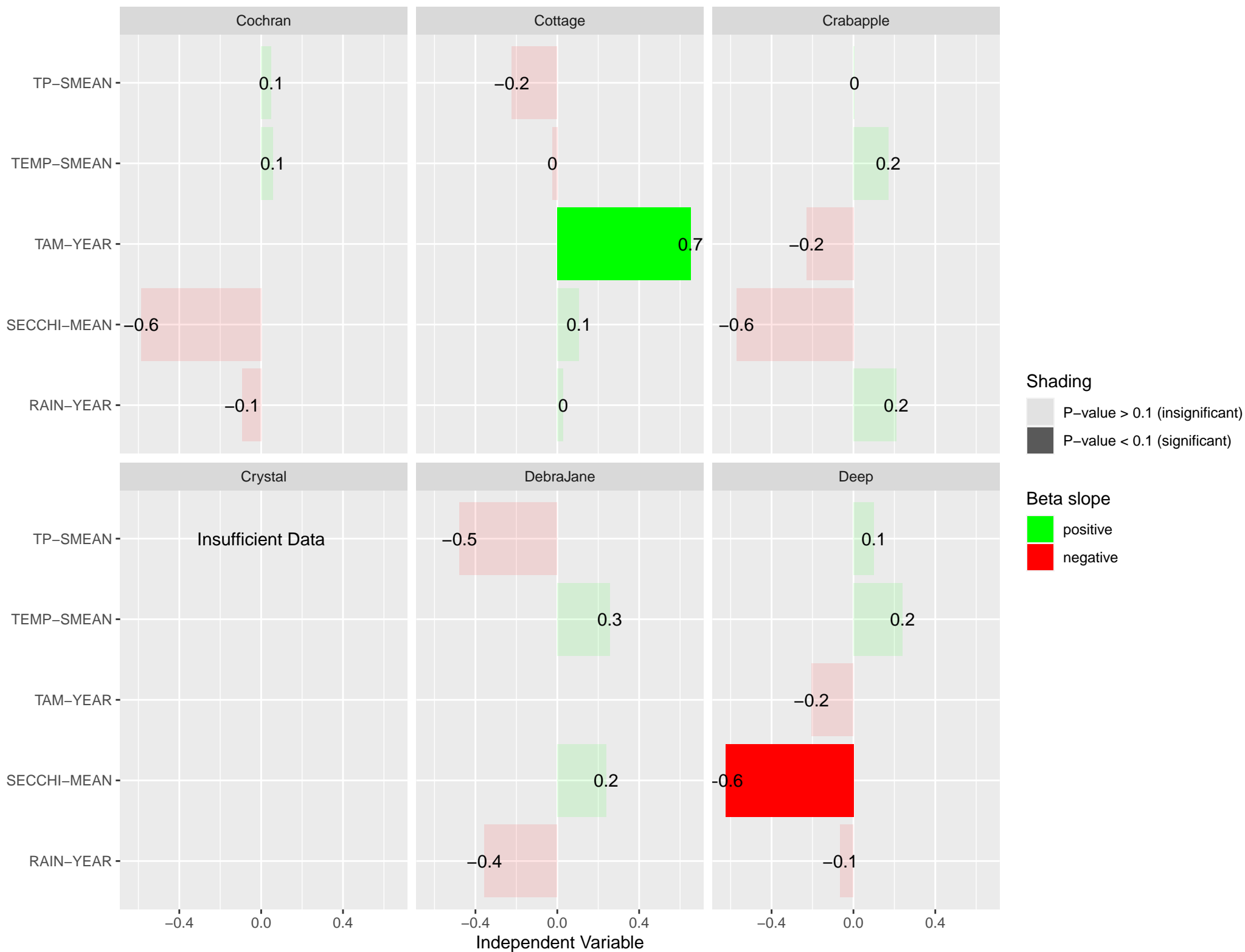
Multiple Regression Results for CHLA-SMAX (Annual data)



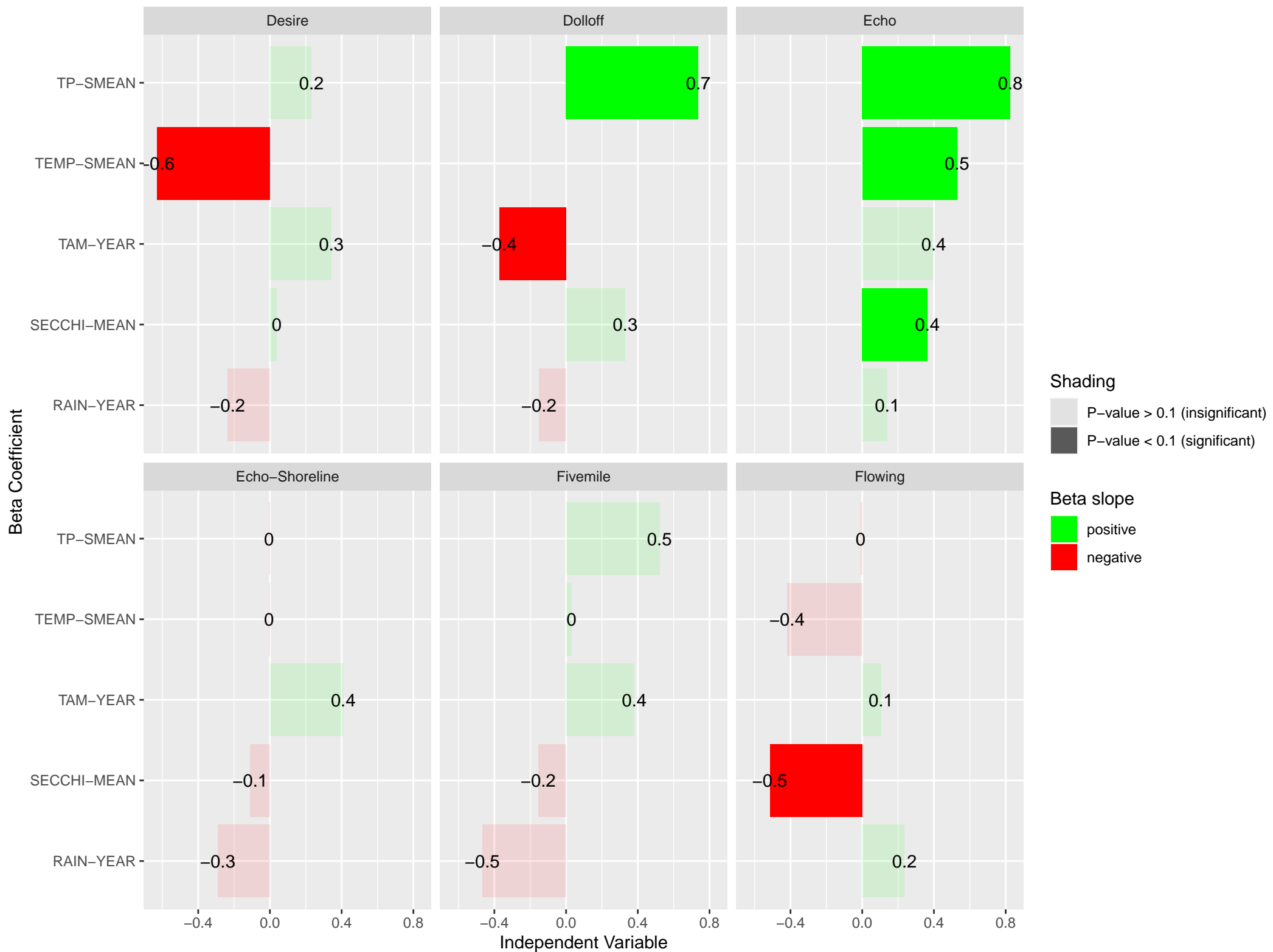
Multiple Regression Results for CHLA-SMAX (Annual data)



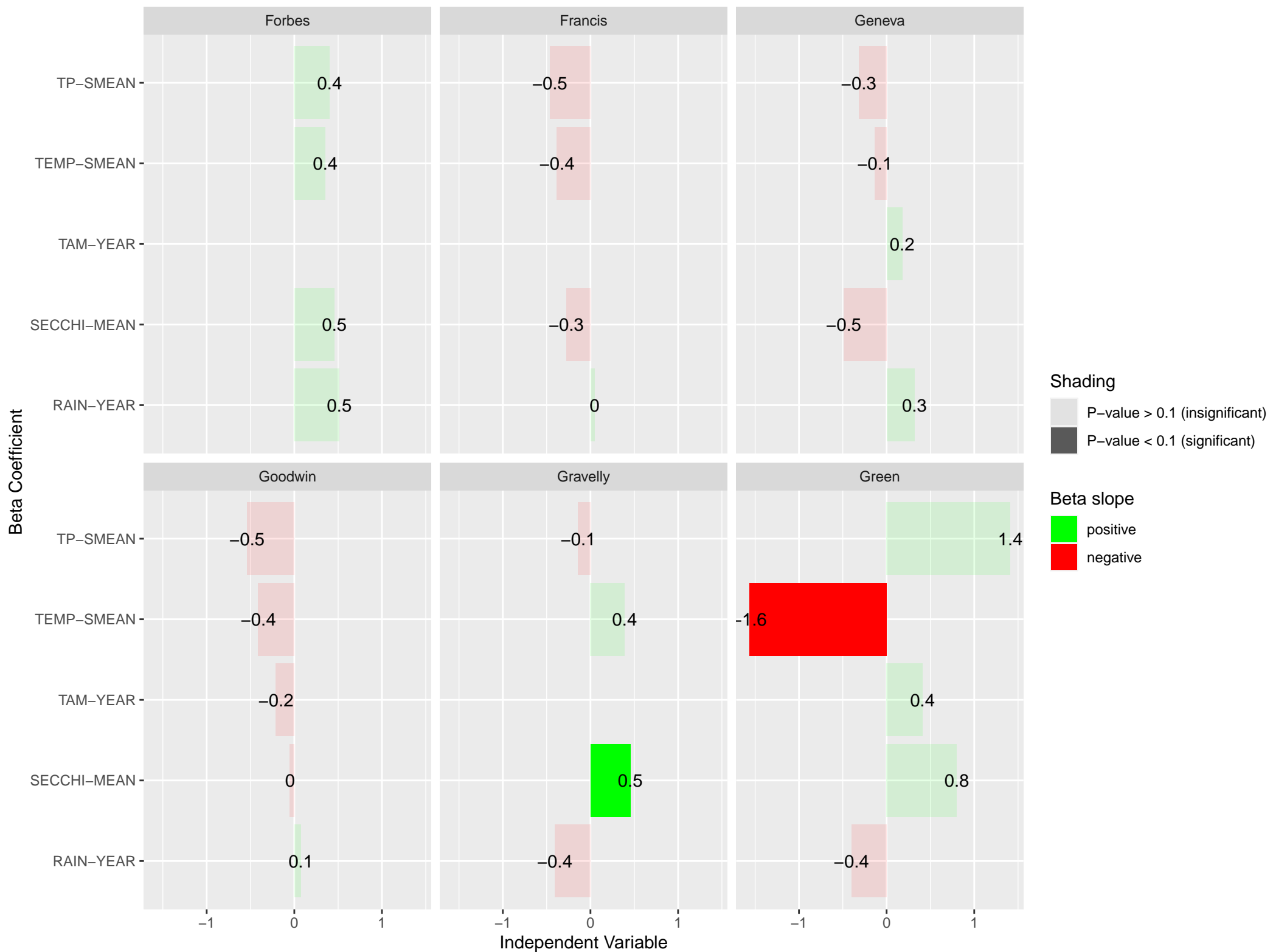
Multiple Regression Results for CHLA-SMAX (Annual data)



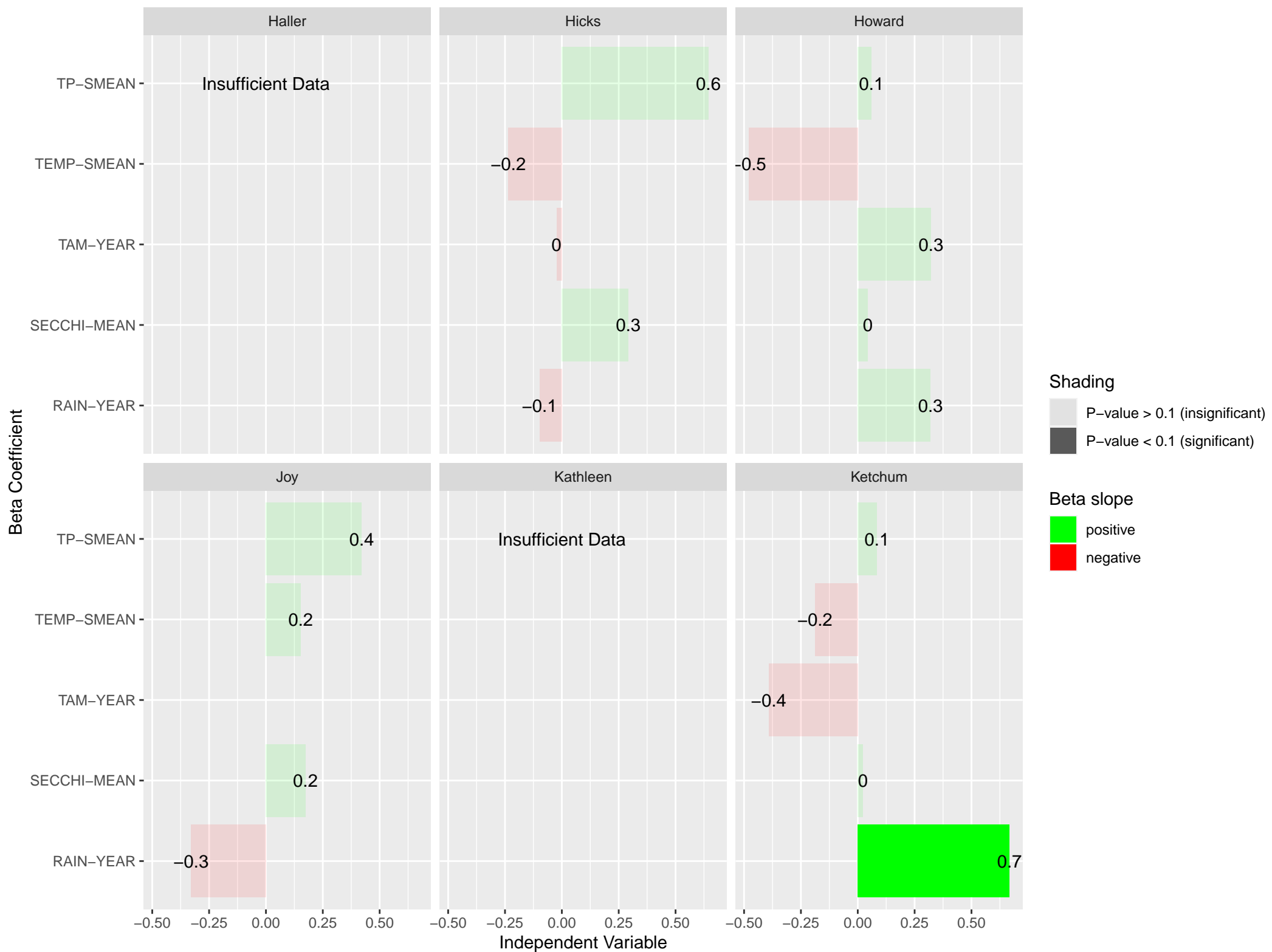
Multiple Regression Results for CHLA-SMAX (Annual data)



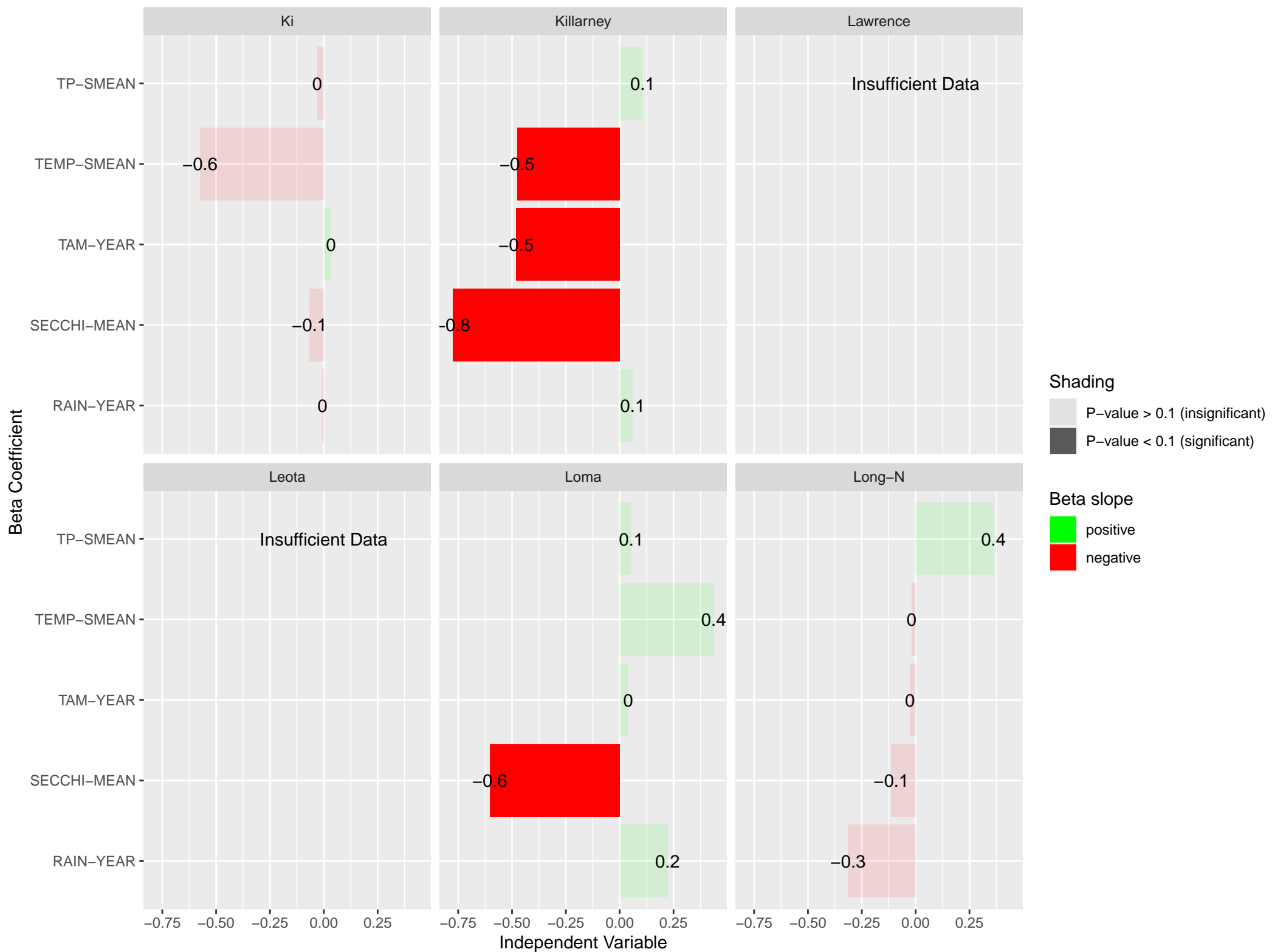
Multiple Regression Results for CHLA-SMAX (Annual data)



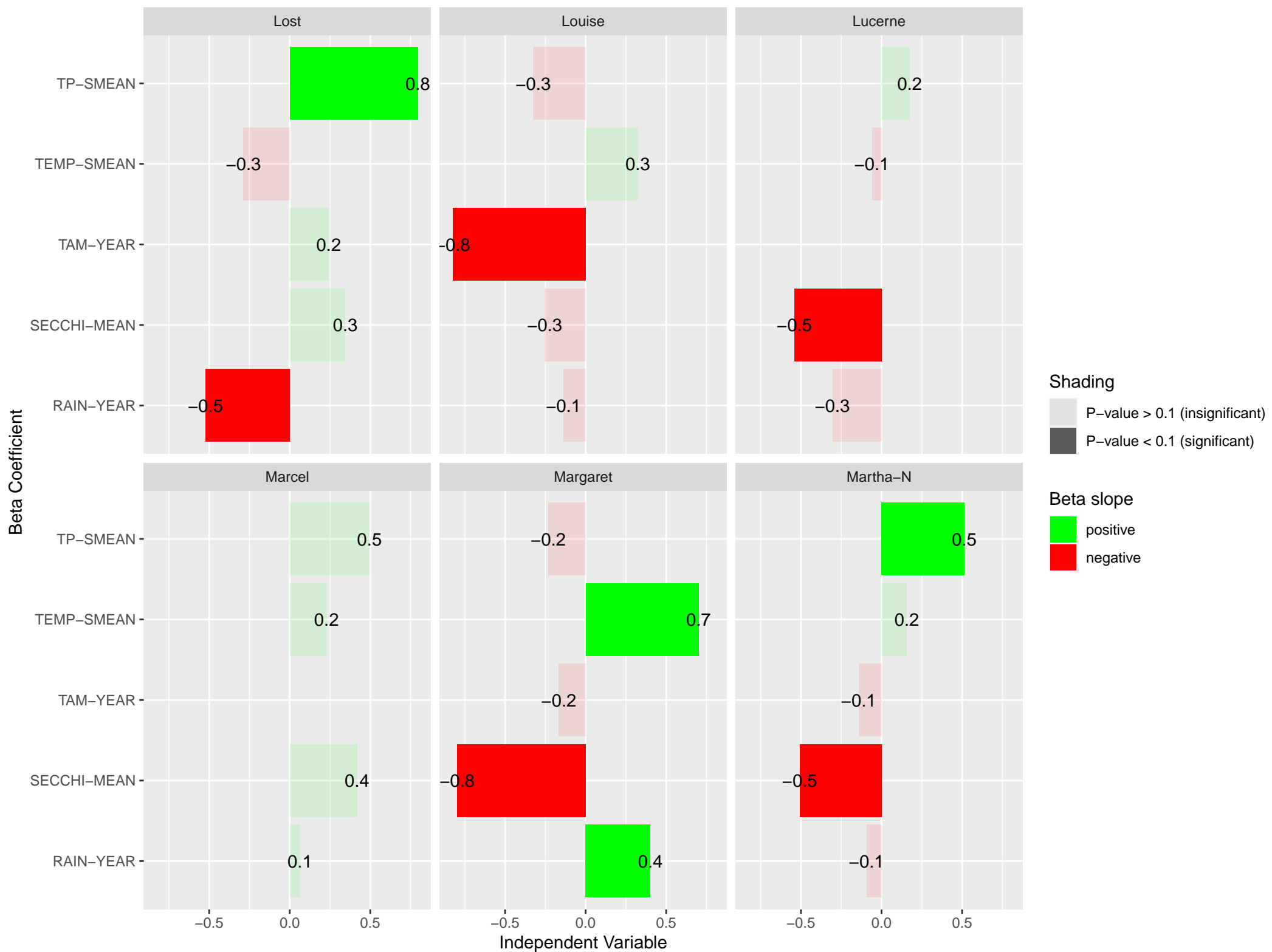
Multiple Regression Results for CHLA-SMAX (Annual data)



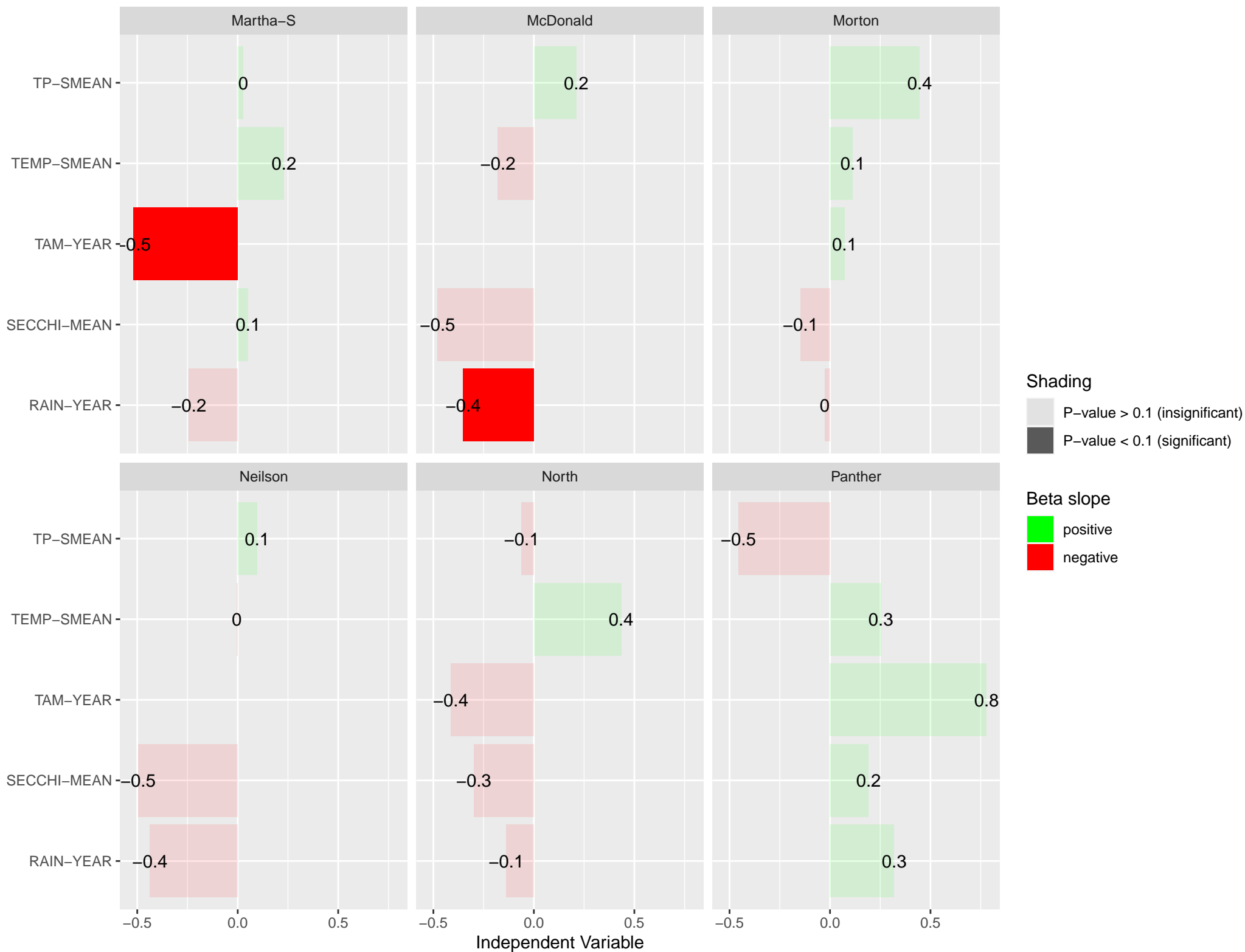
Multiple Regression Results for CHLA-SMAX (Annual data)



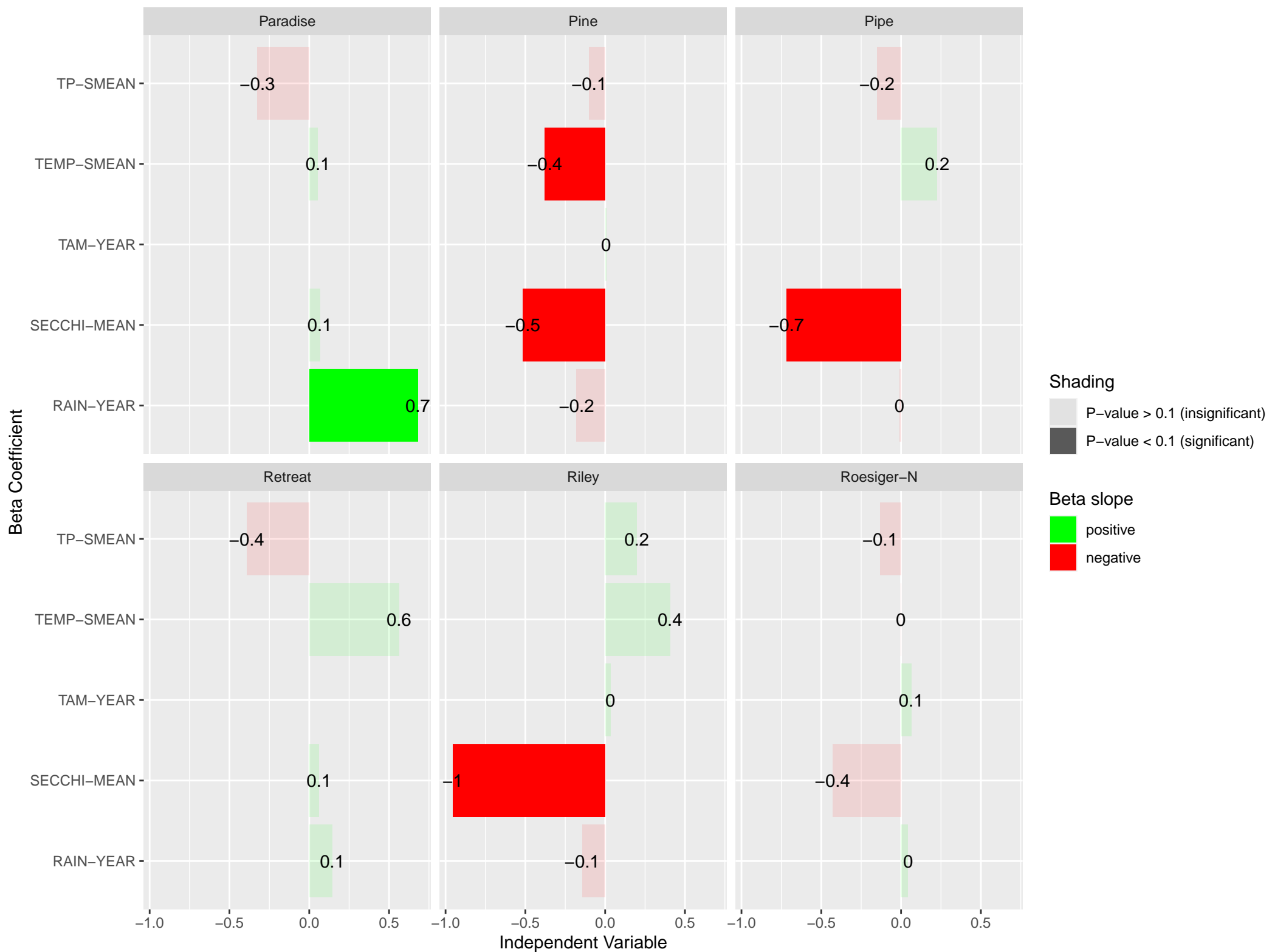
Multiple Regression Results for CHLA-SMAX (Annual data)



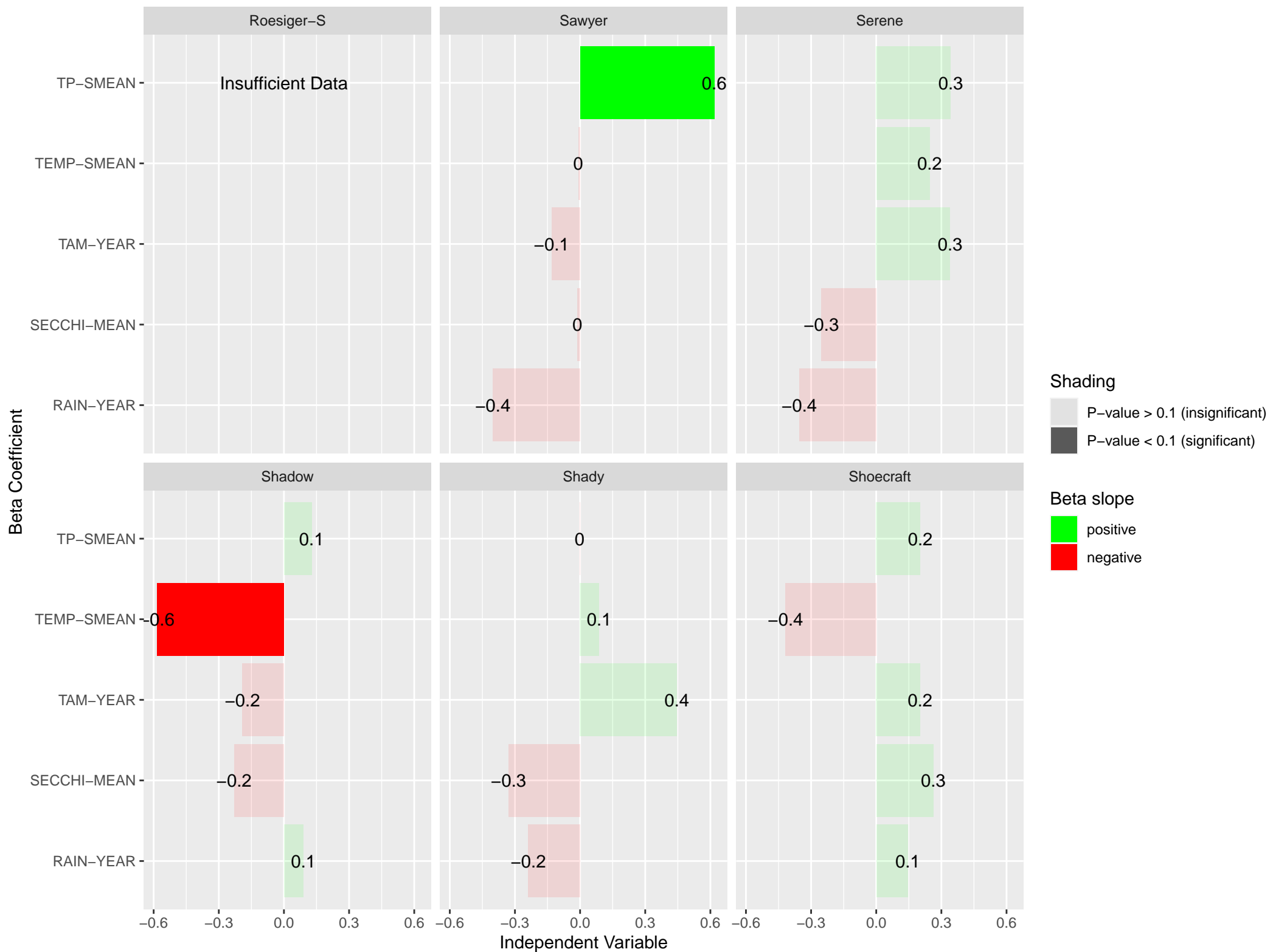
Multiple Regression Results for CHLA-SMAX (Annual data)



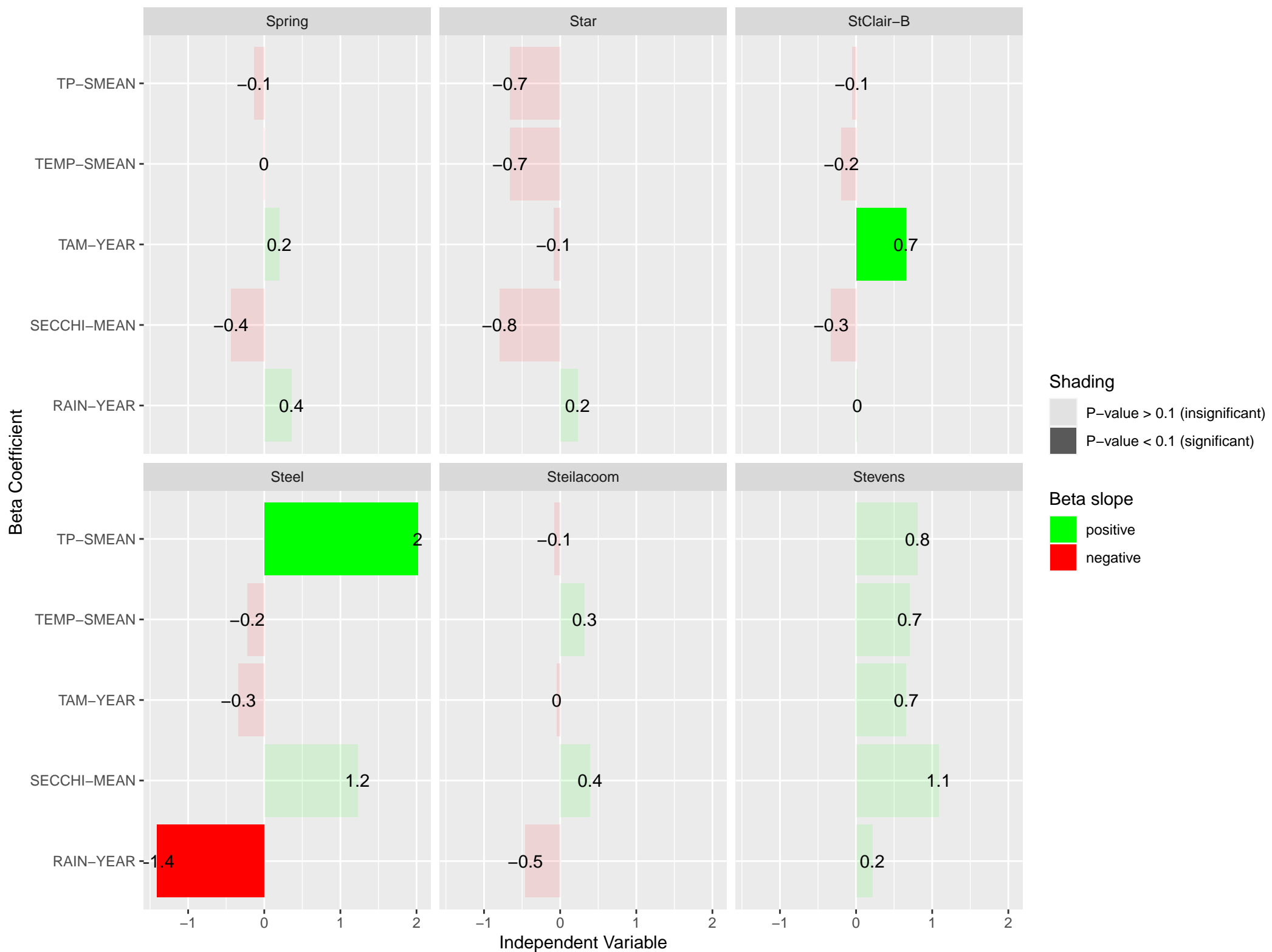
Multiple Regression Results for CHLA-SMAX (Annual data)



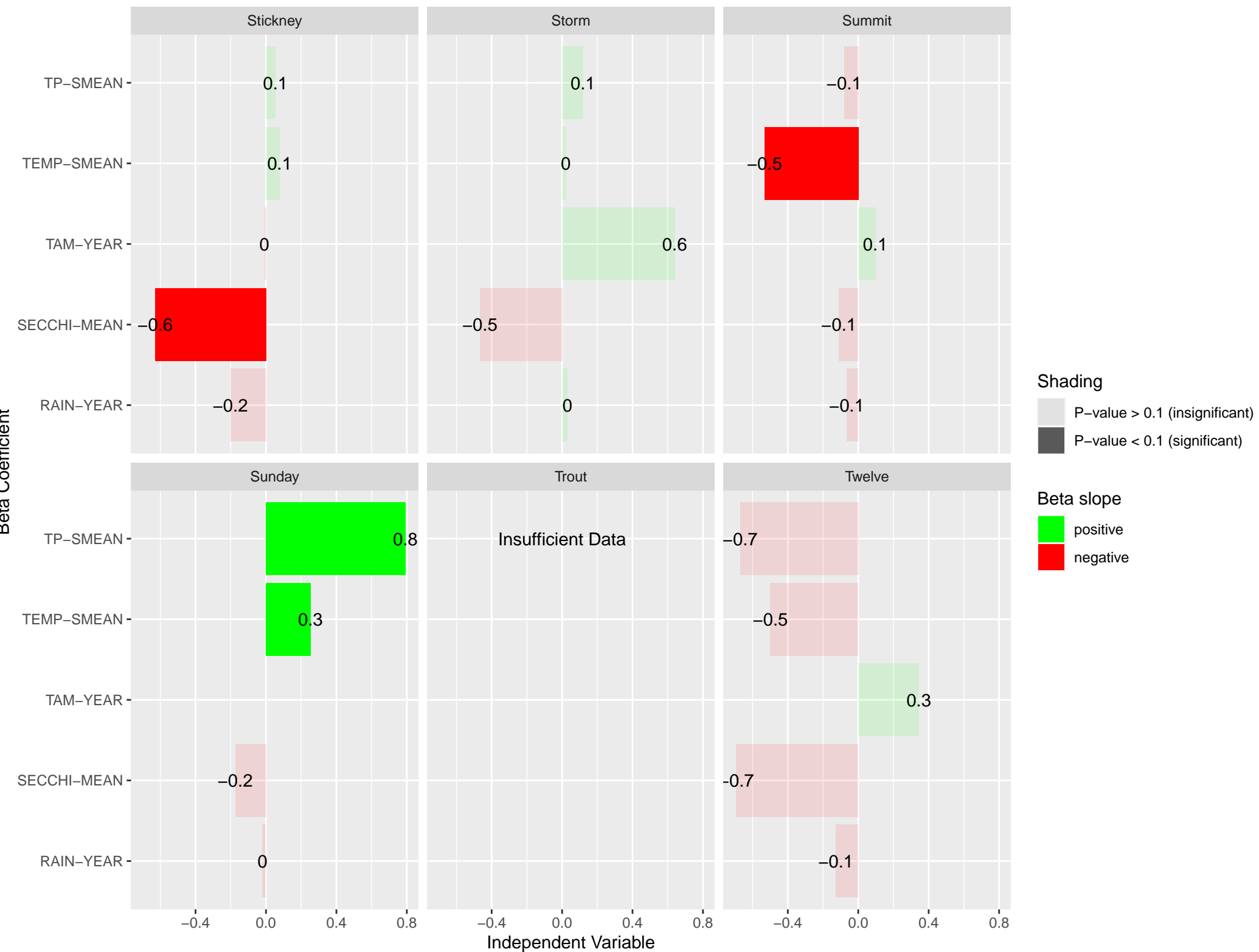
Multiple Regression Results for CHLA-SMAX (Annual data)



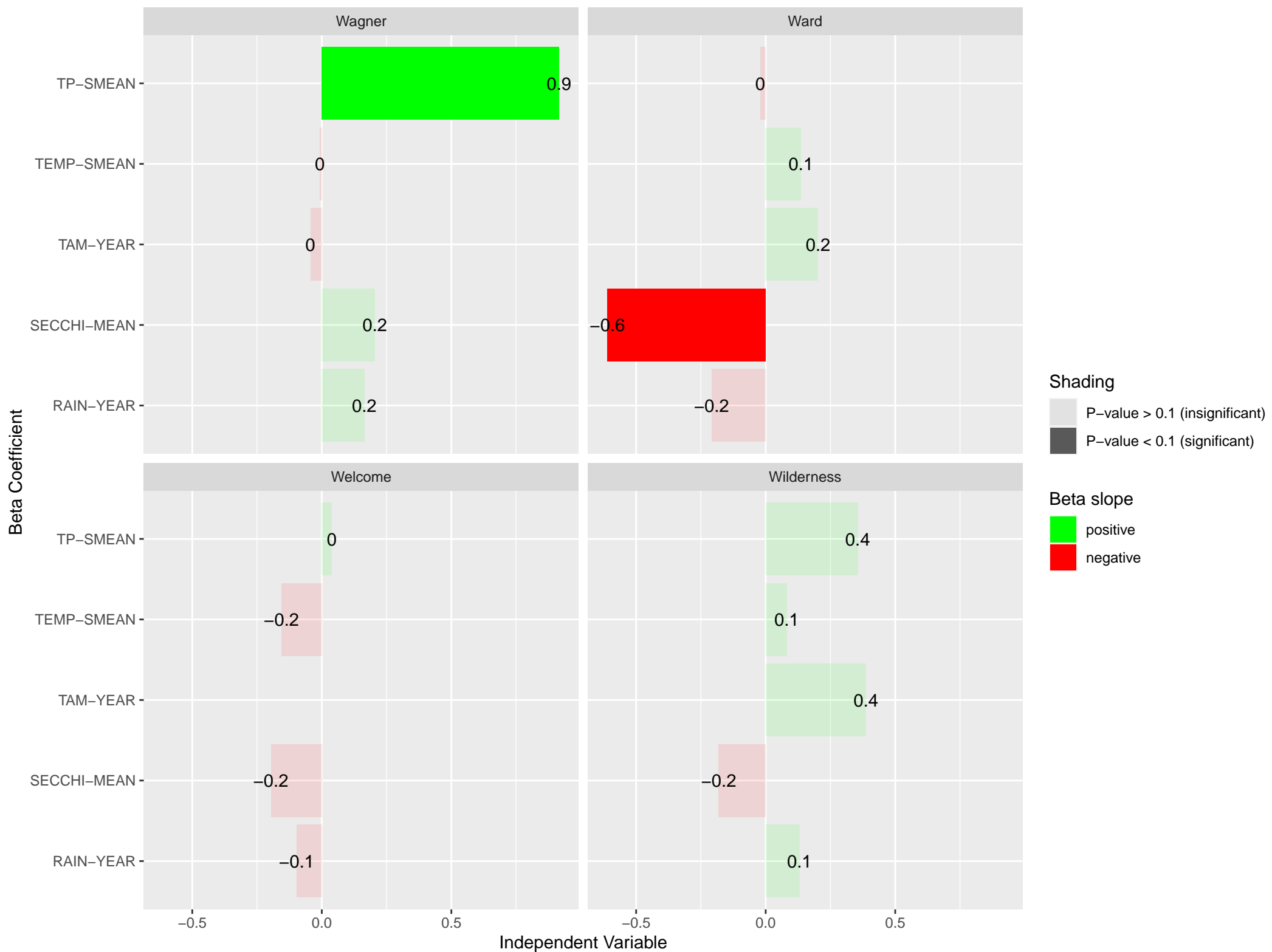
Multiple Regression Results for CHLA-SMAX (Annual data)



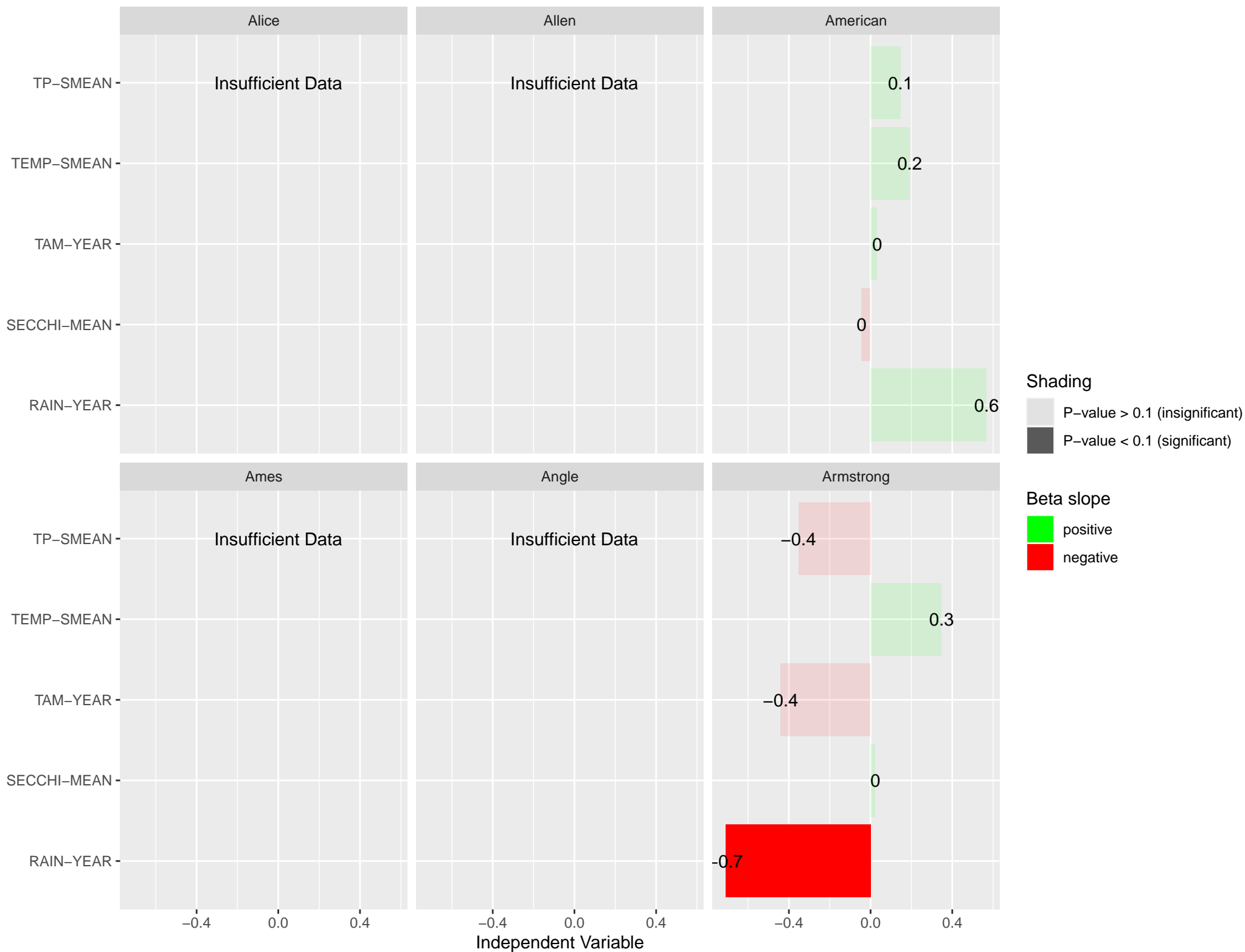
Multiple Regression Results for CHLA-SMAX (Annual data)



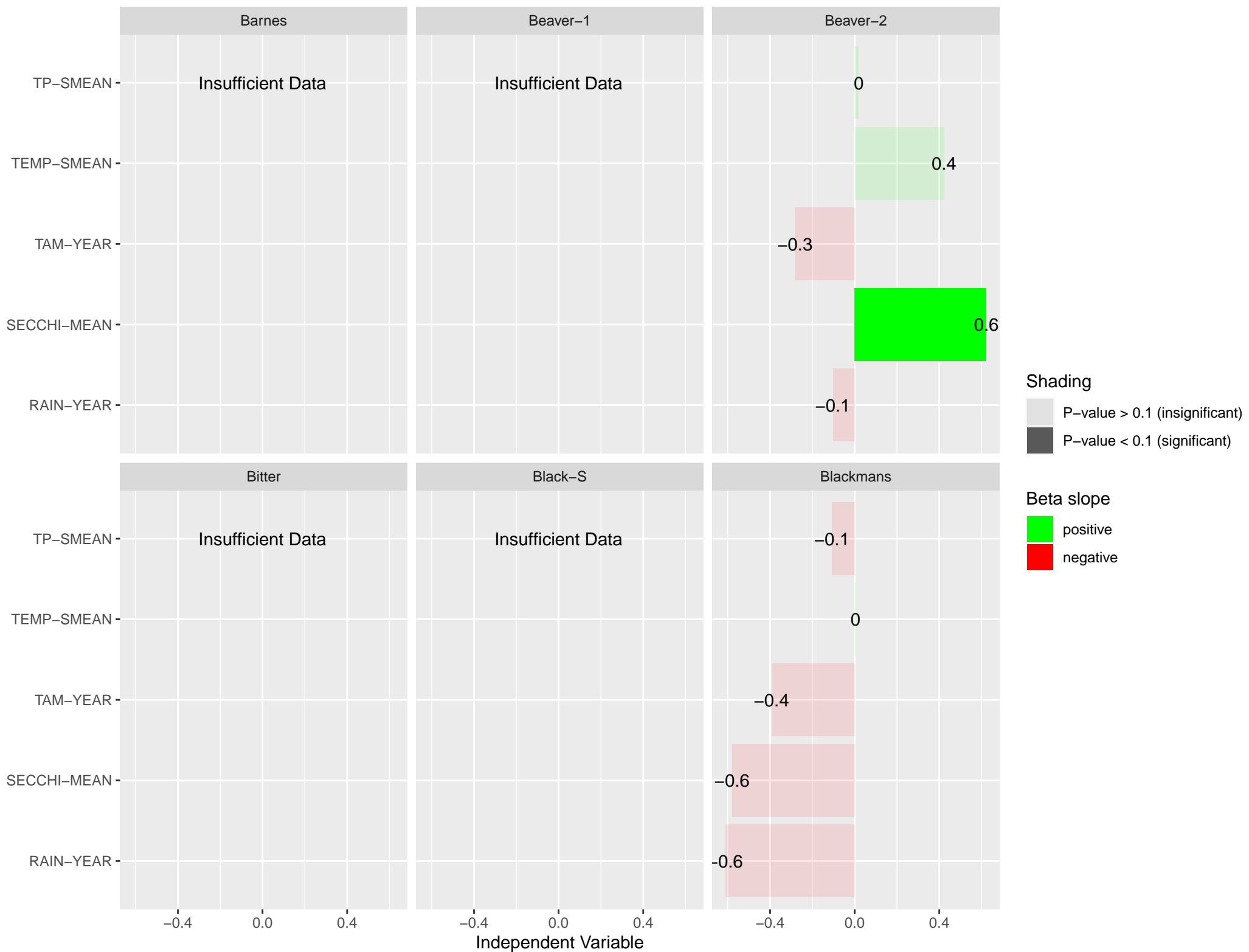
Multiple Regression Results for CHLA-SMAX (Annual data)



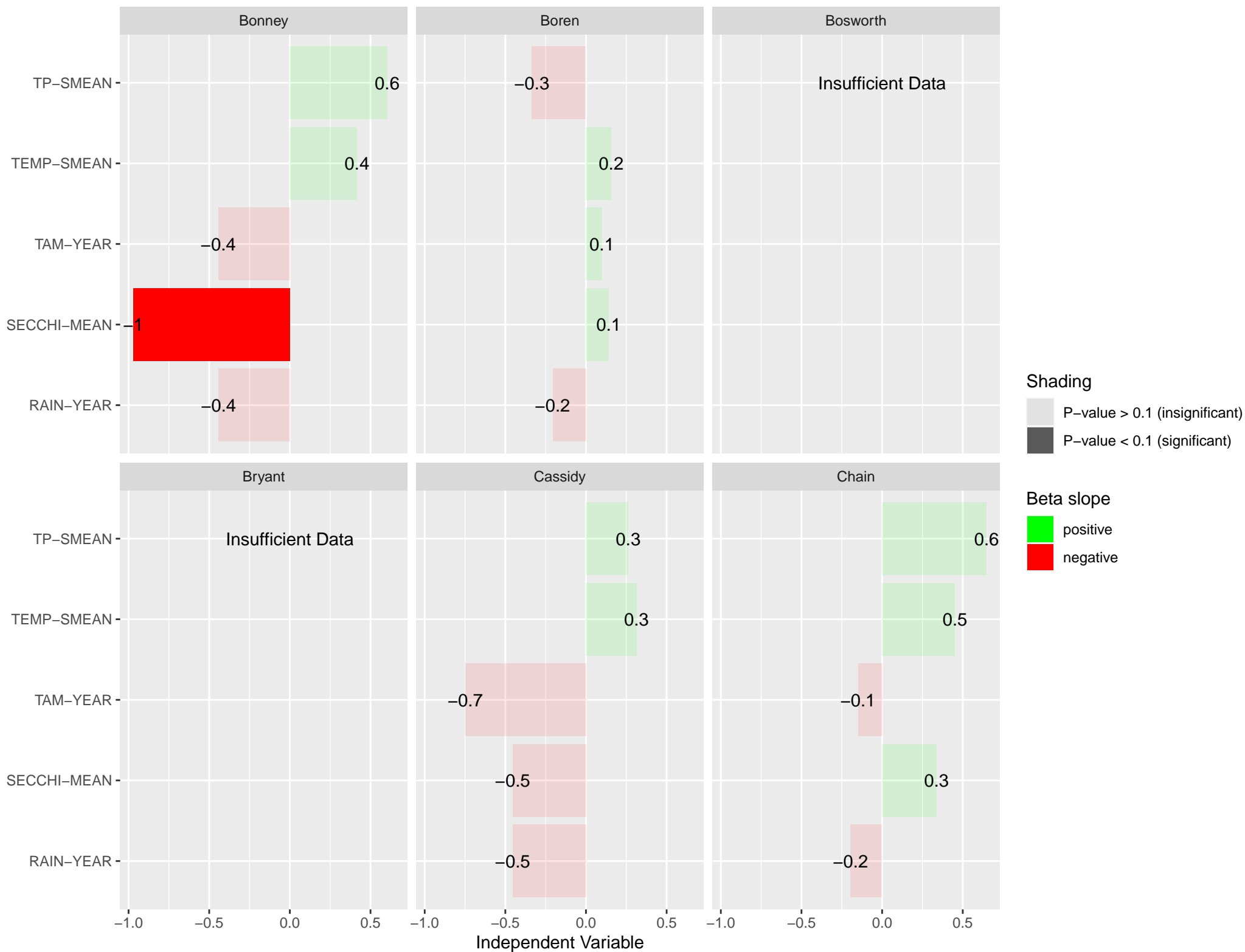
Multiple Regression Results for MC-MEAN (Annual data)



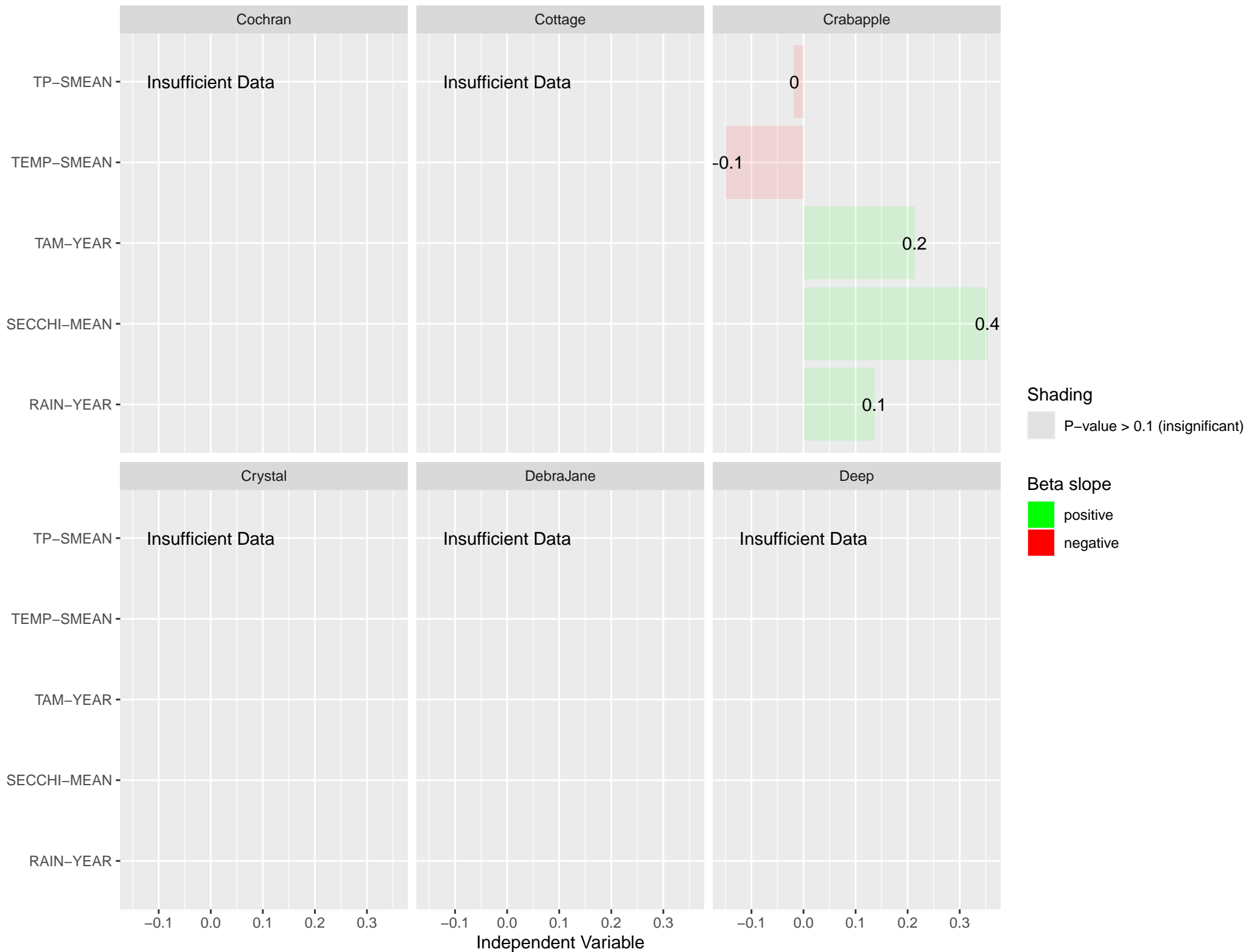
Multiple Regression Results for MC-MEAN (Annual data)



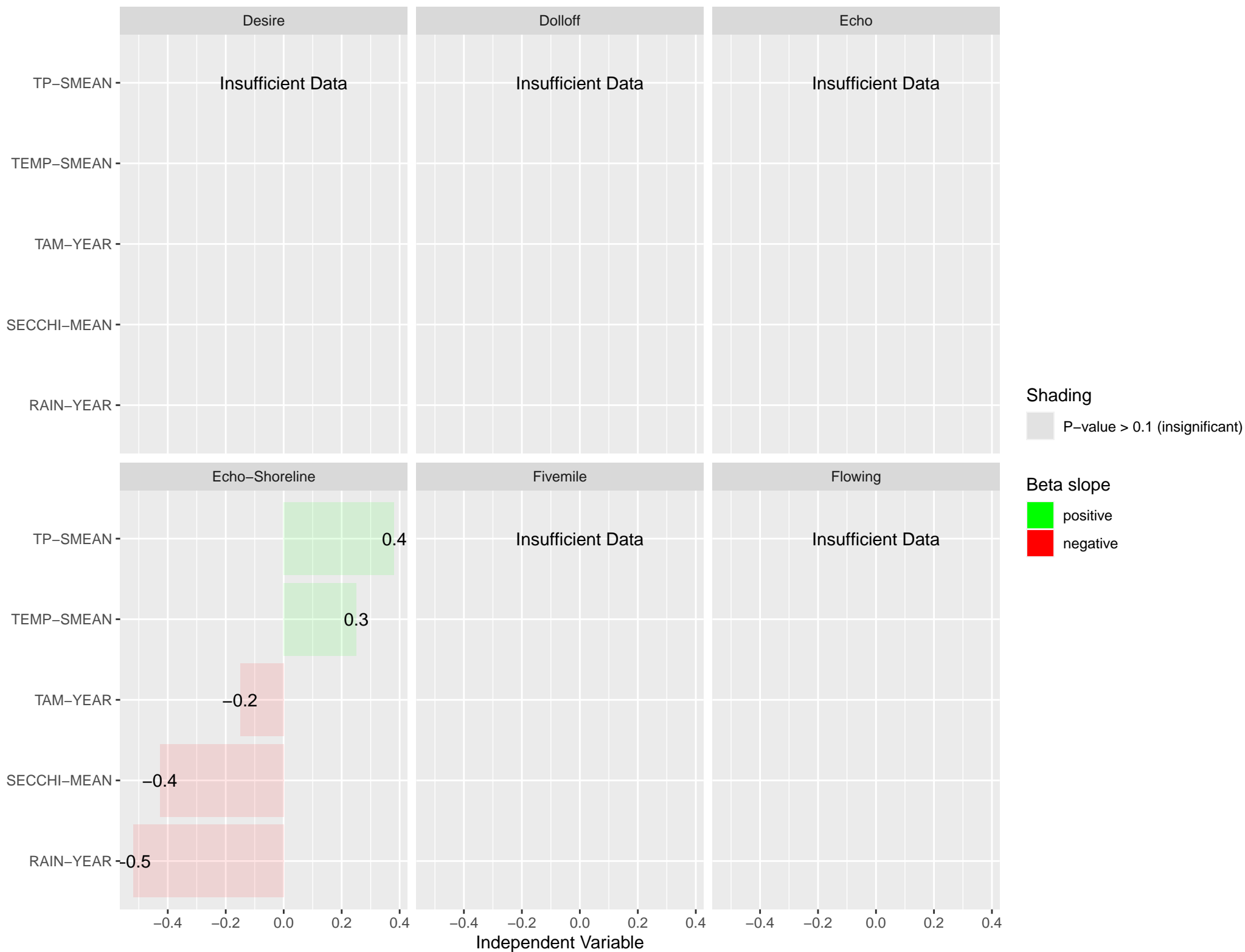
Multiple Regression Results for MC-MEAN (Annual data)



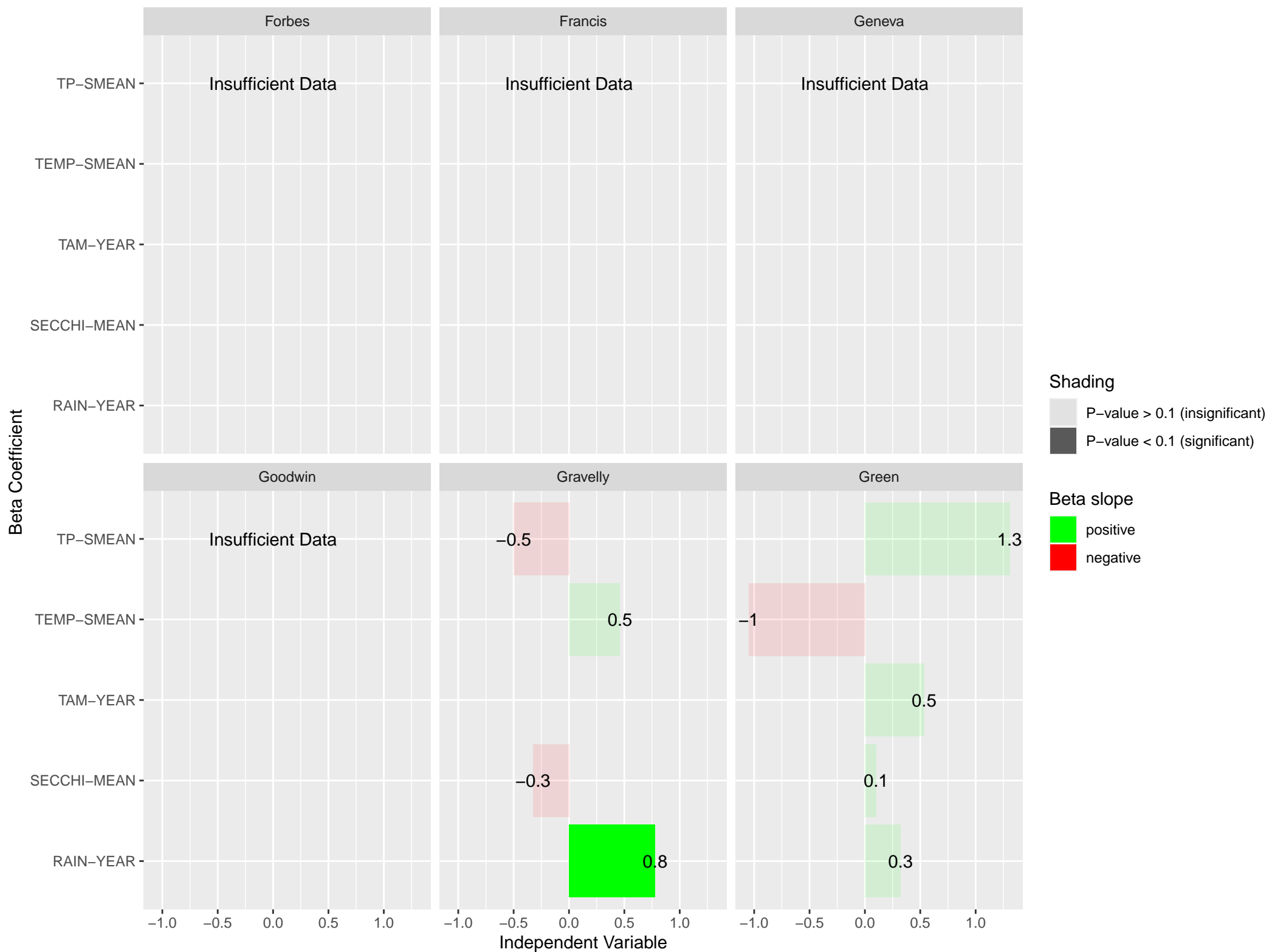
Multiple Regression Results for MC-MEAN (Annual data)



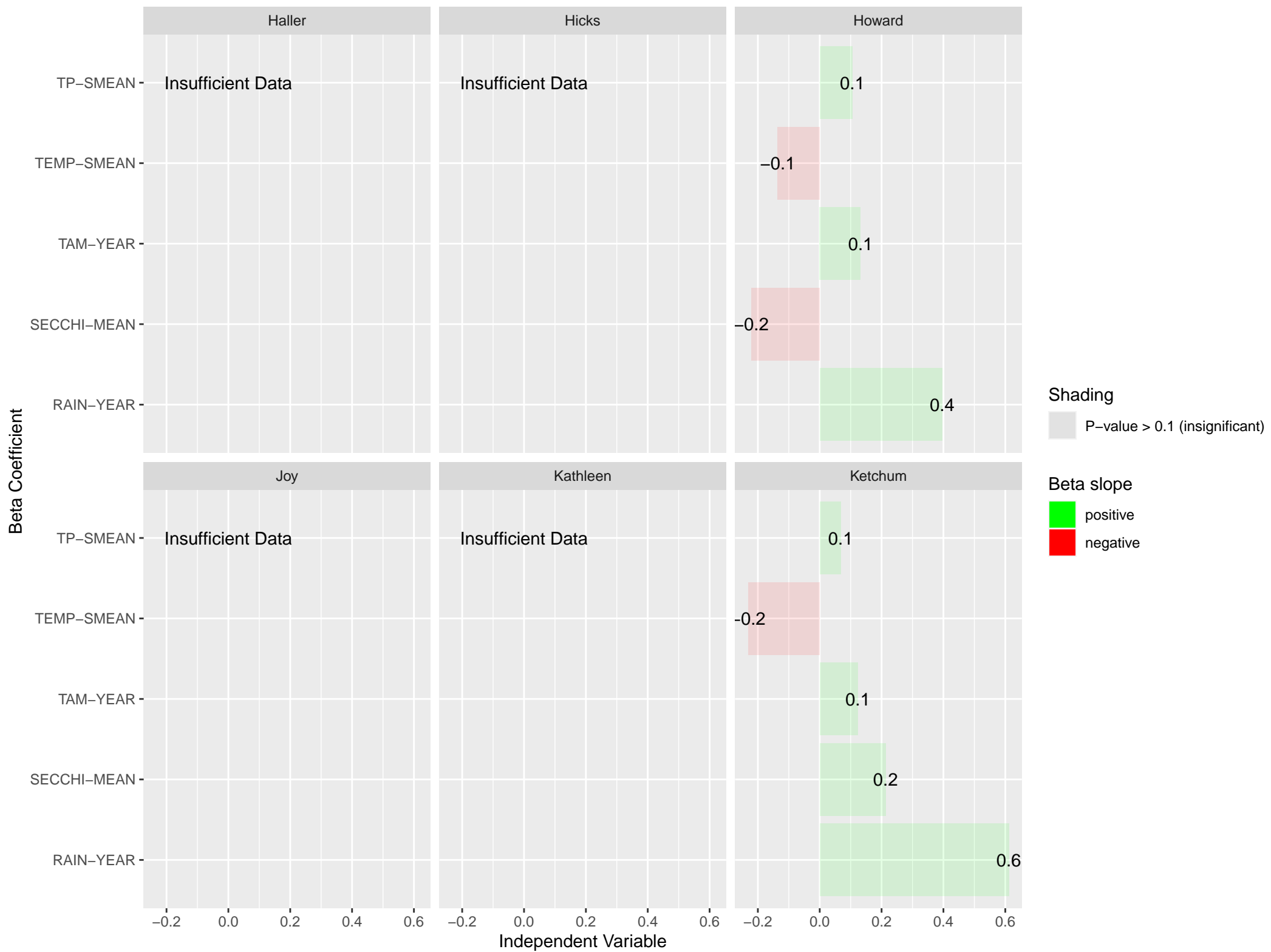
Multiple Regression Results for MC-MEAN (Annual data)



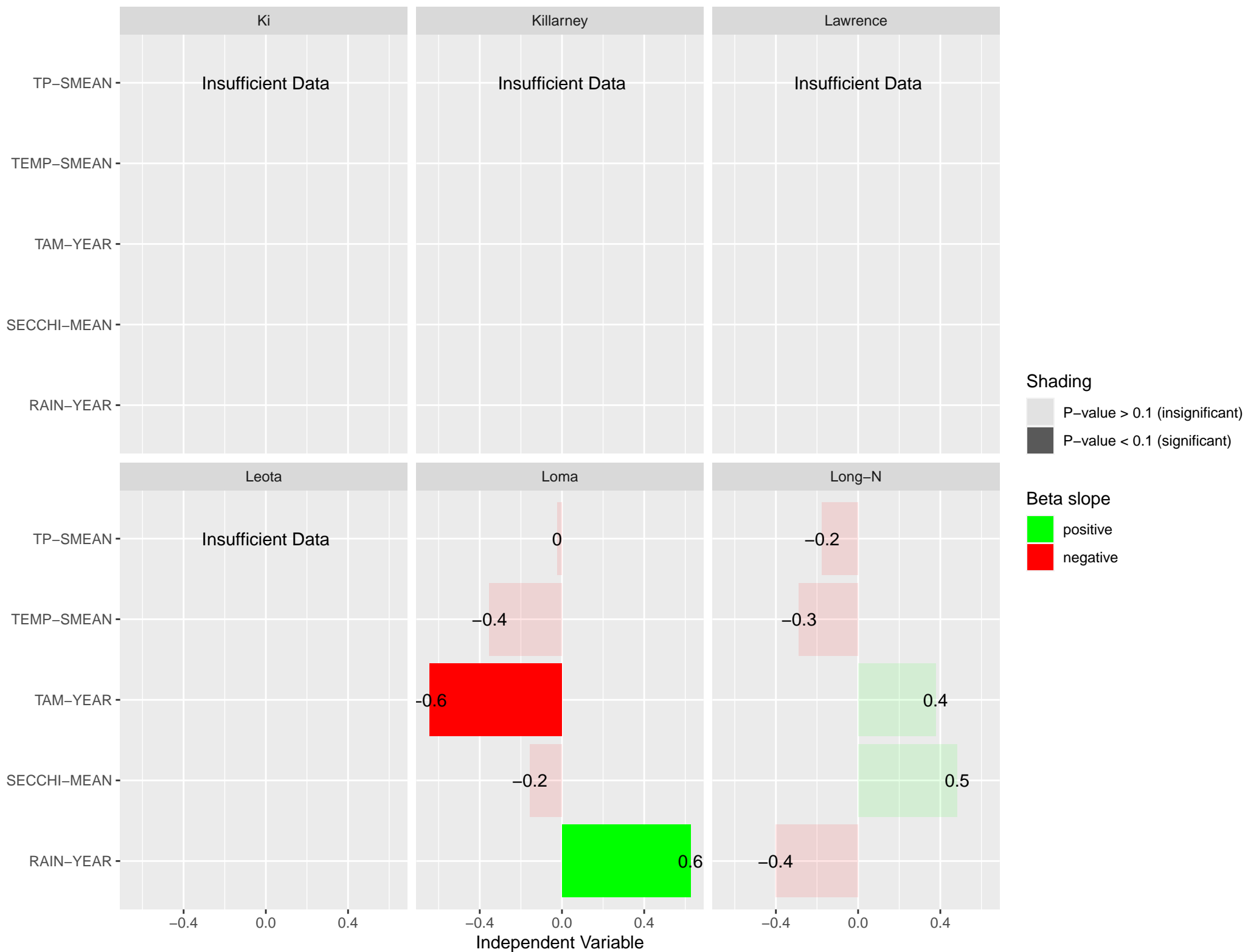
Multiple Regression Results for MC-MEAN (Annual data)



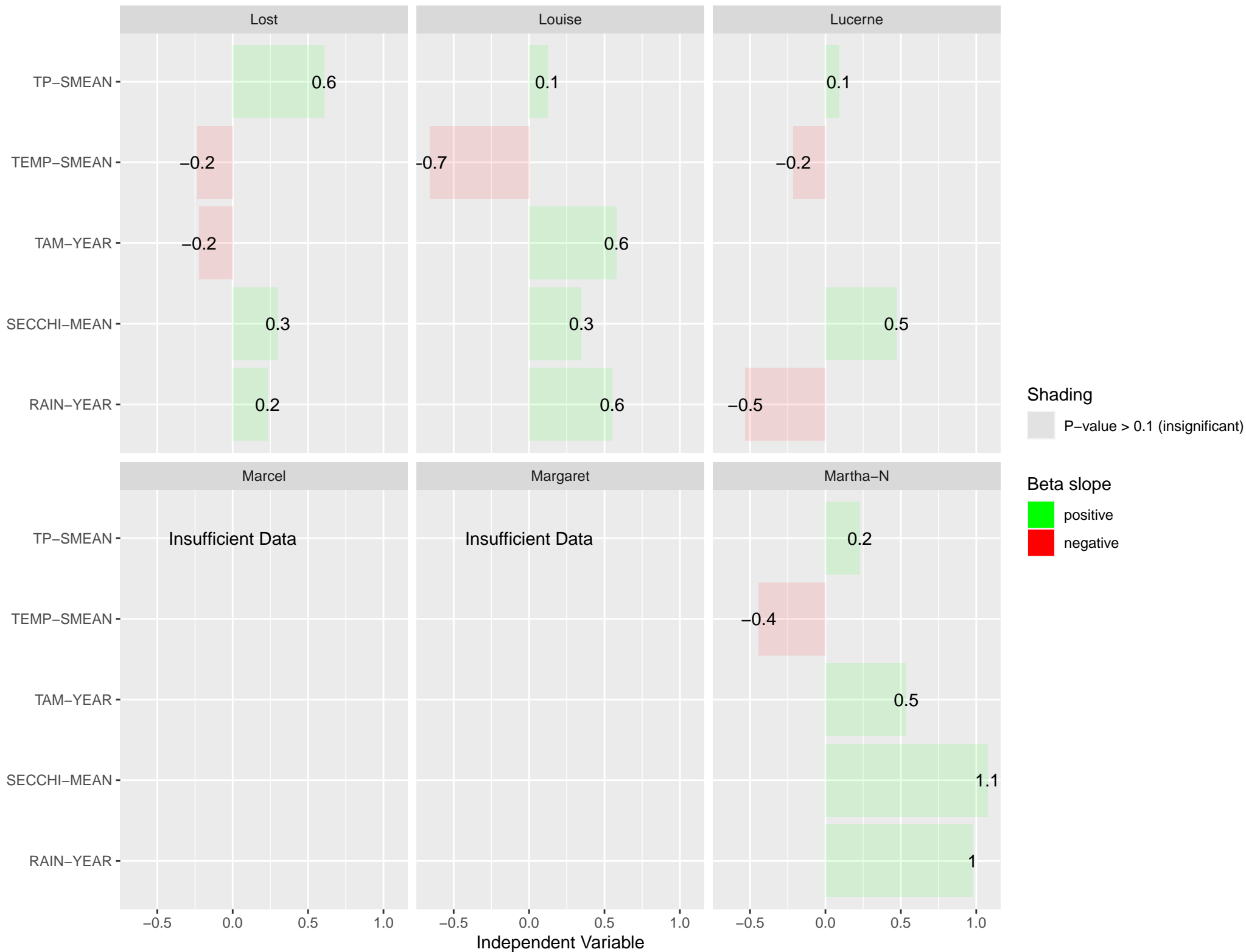
Multiple Regression Results for MC-MEAN (Annual data)



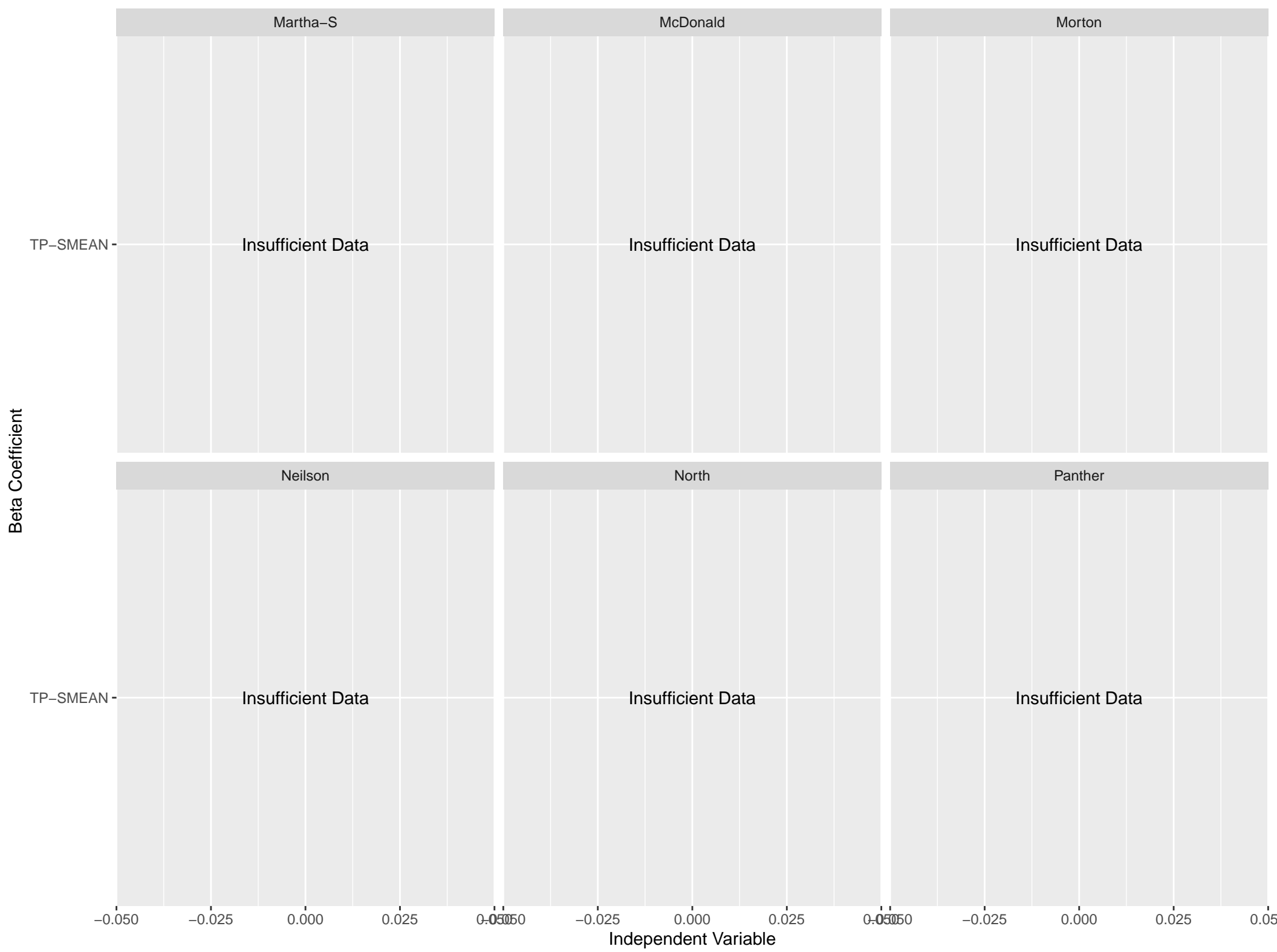
Multiple Regression Results for MC-MEAN (Annual data)



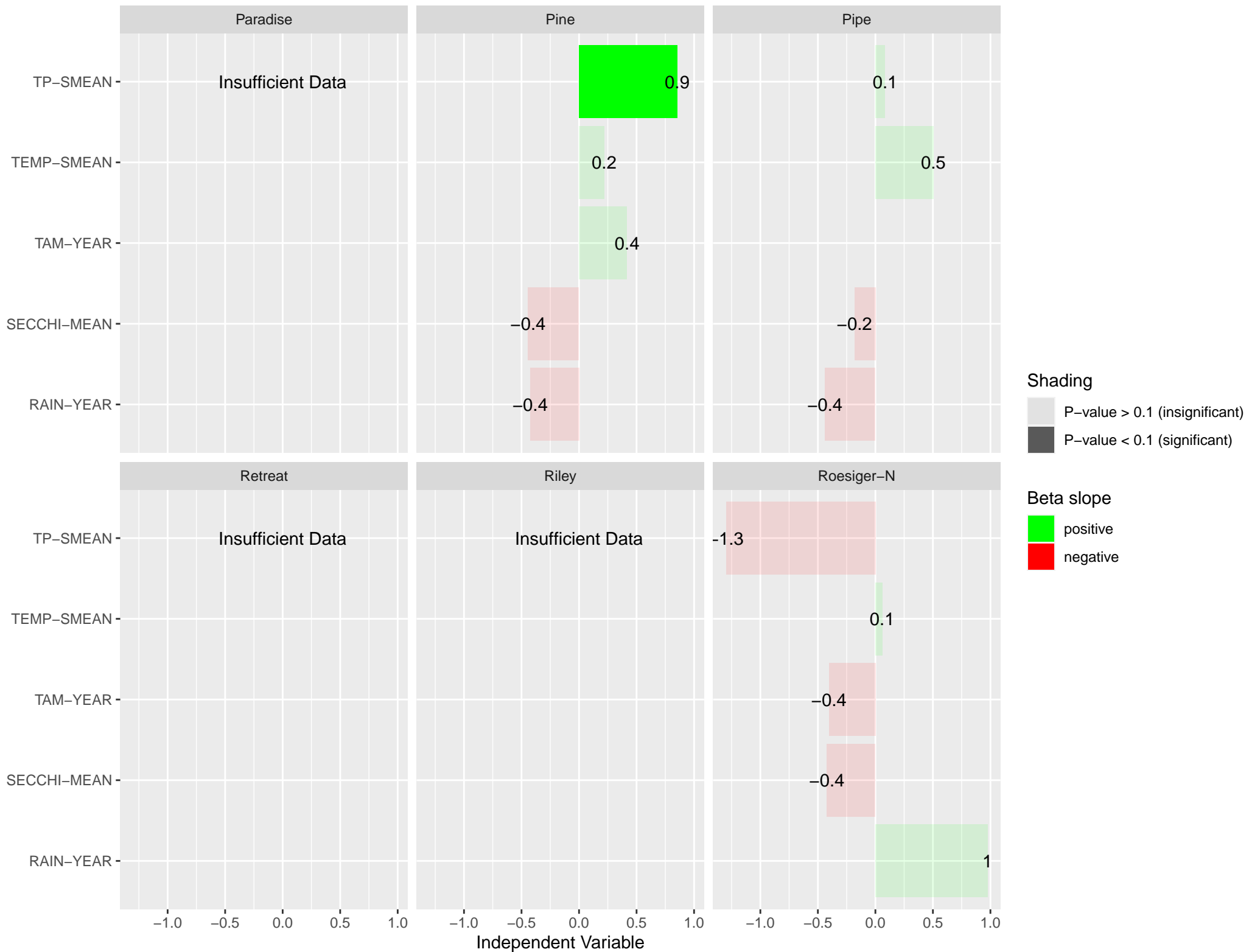
Multiple Regression Results for MC-MEAN (Annual data)



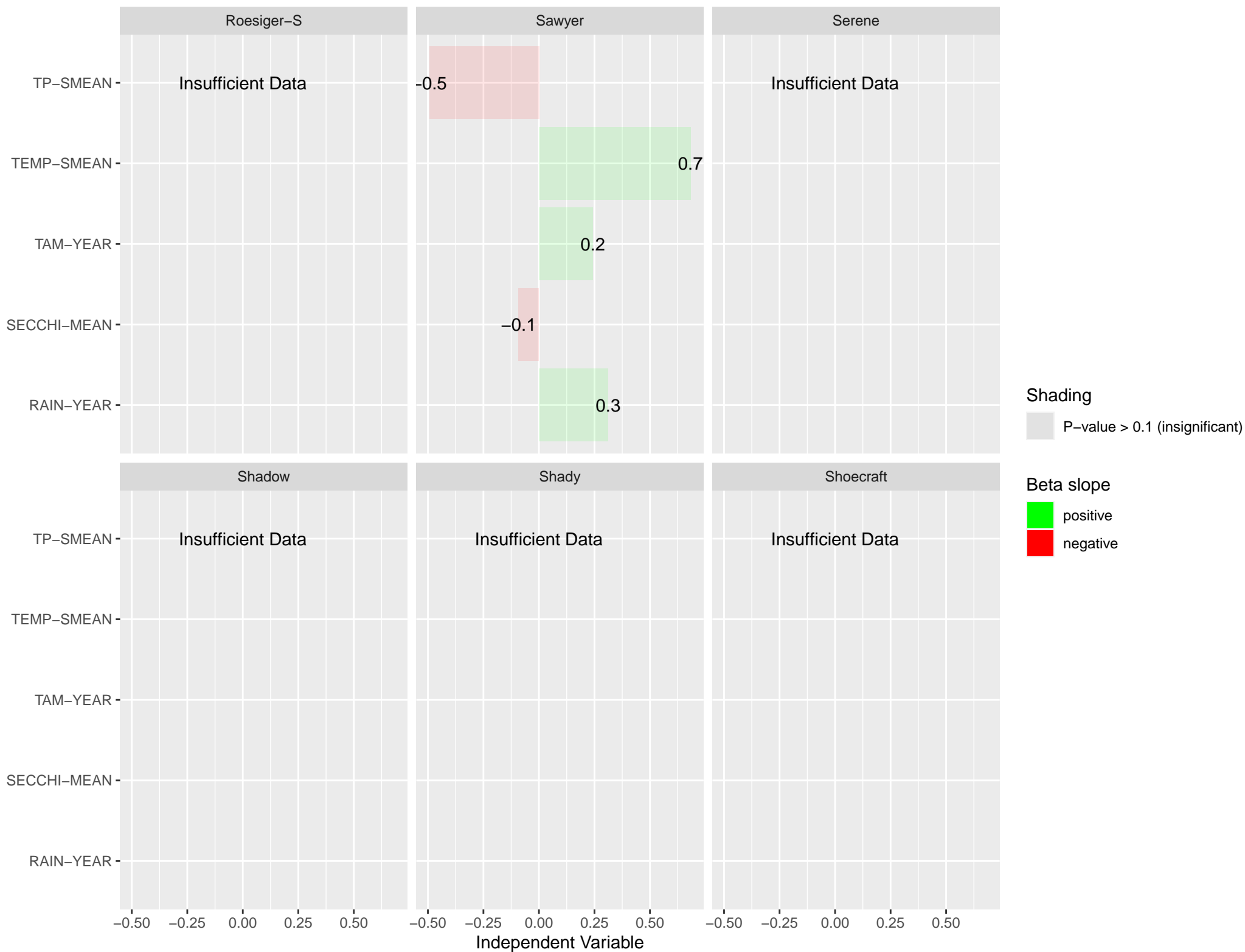
Multiple Regression Results for MC-MEAN (Annual data)



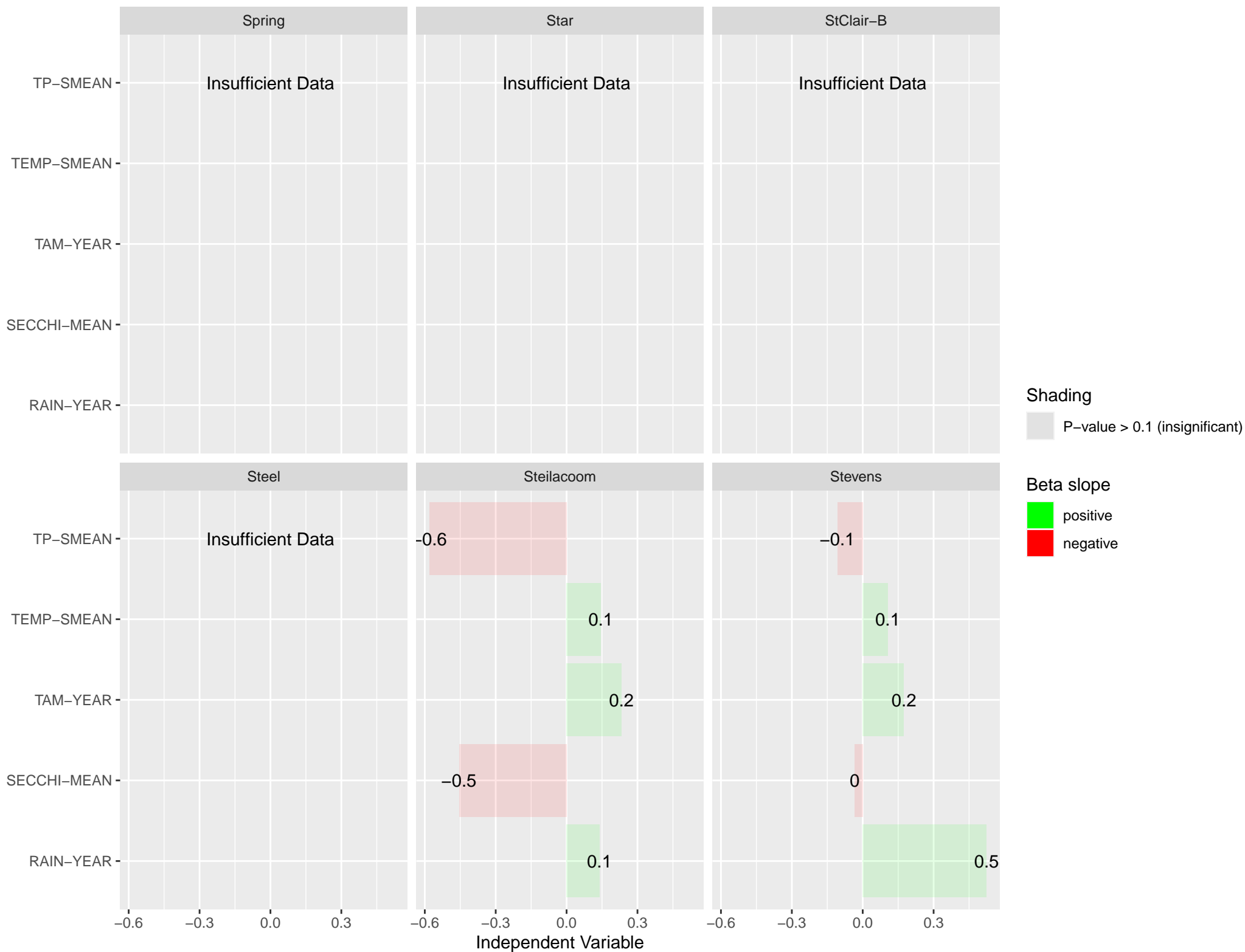
Multiple Regression Results for MC-MEAN (Annual data)



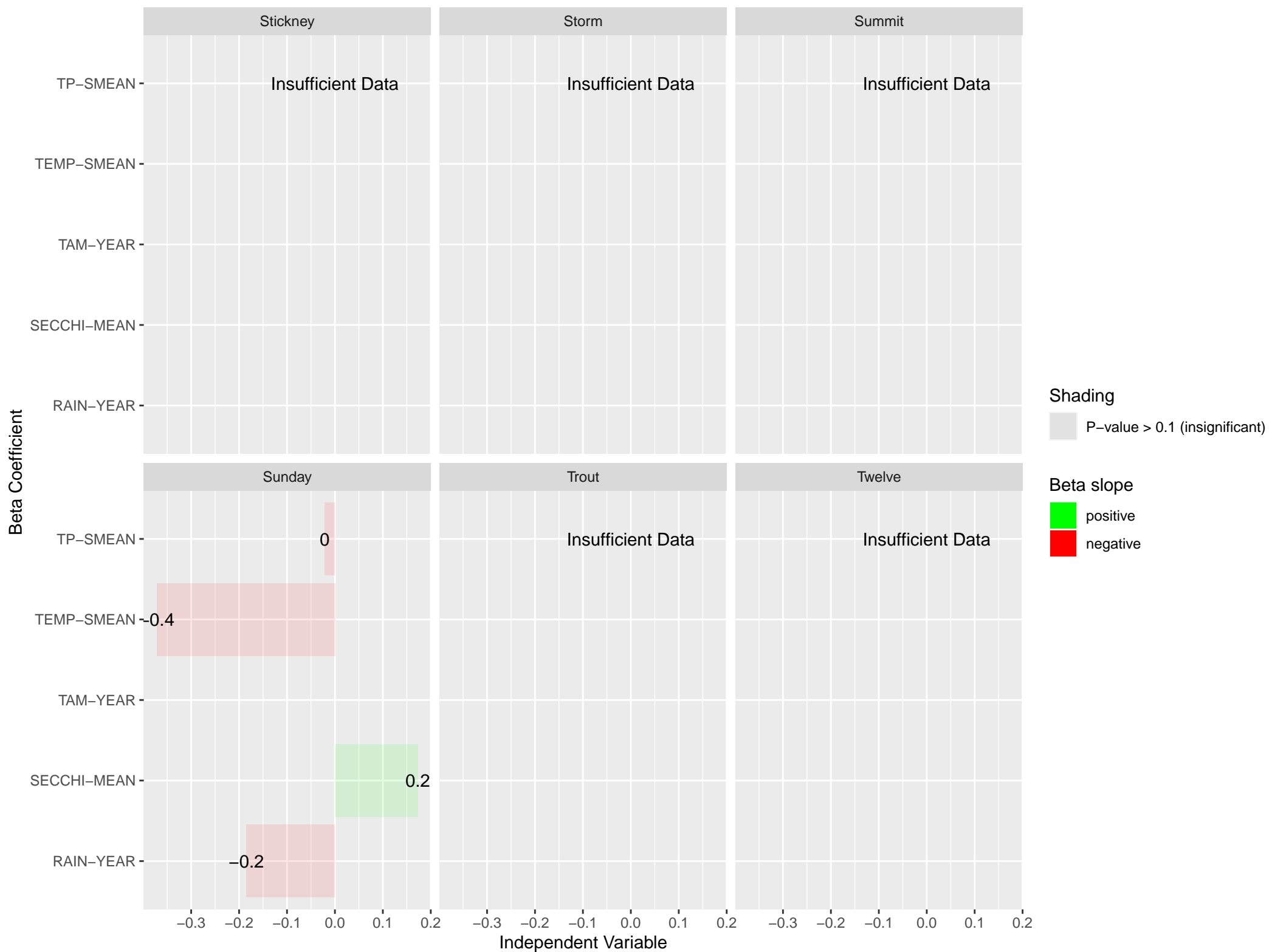
Multiple Regression Results for MC-MEAN (Annual data)



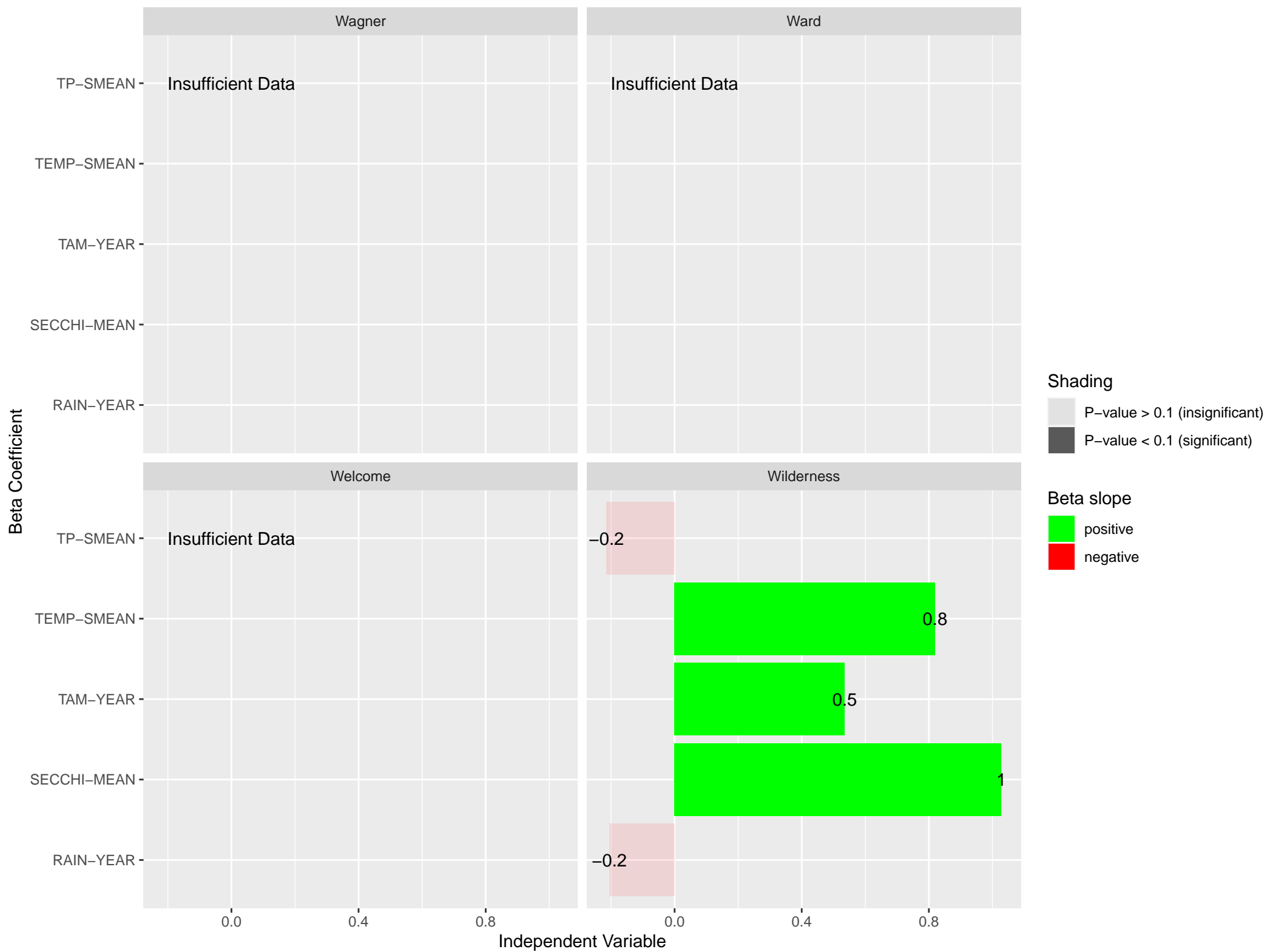
Multiple Regression Results for MC-MEAN (Annual data)



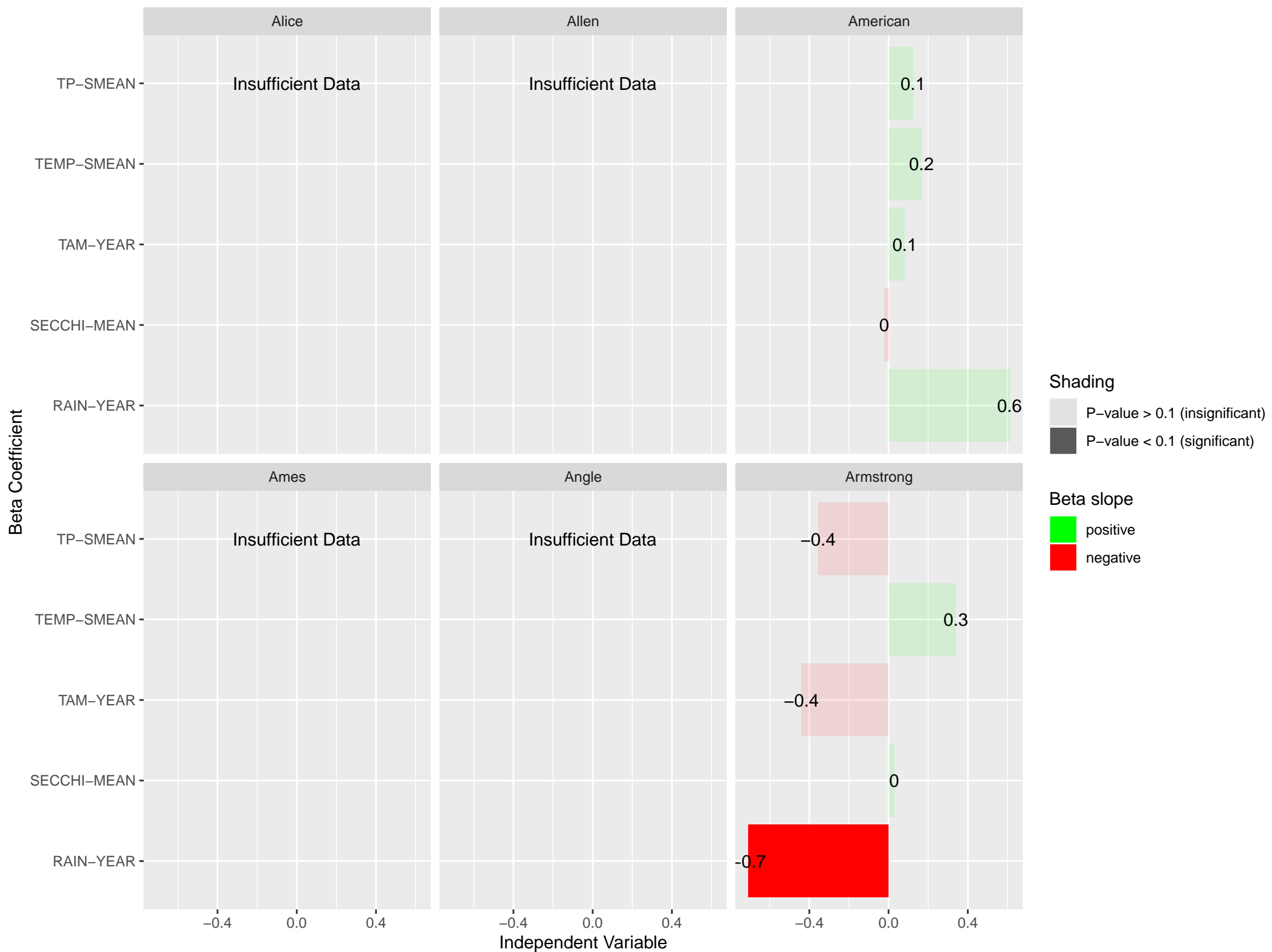
Multiple Regression Results for MC-MEAN (Annual data)



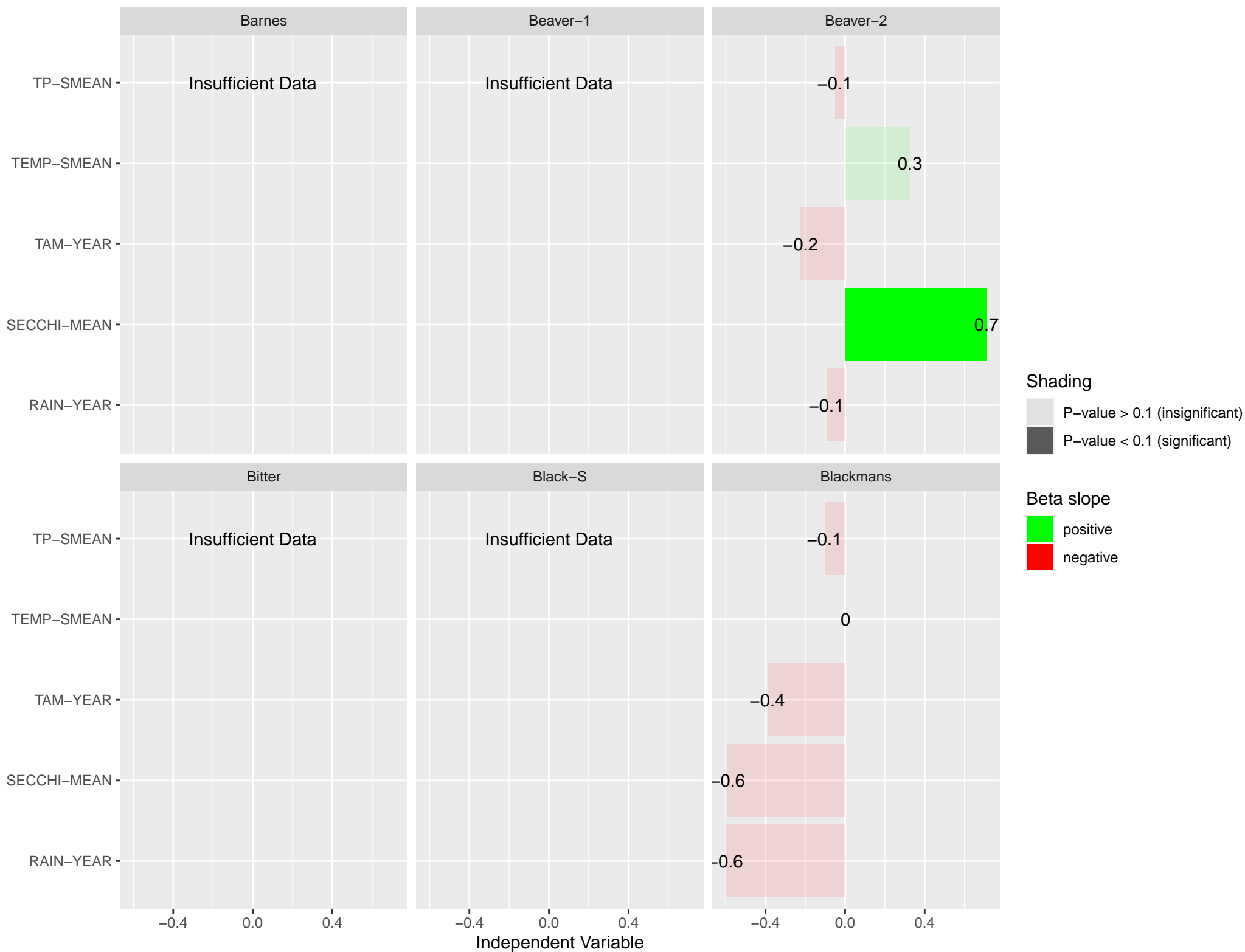
Multiple Regression Results for MC-MEAN (Annual data)



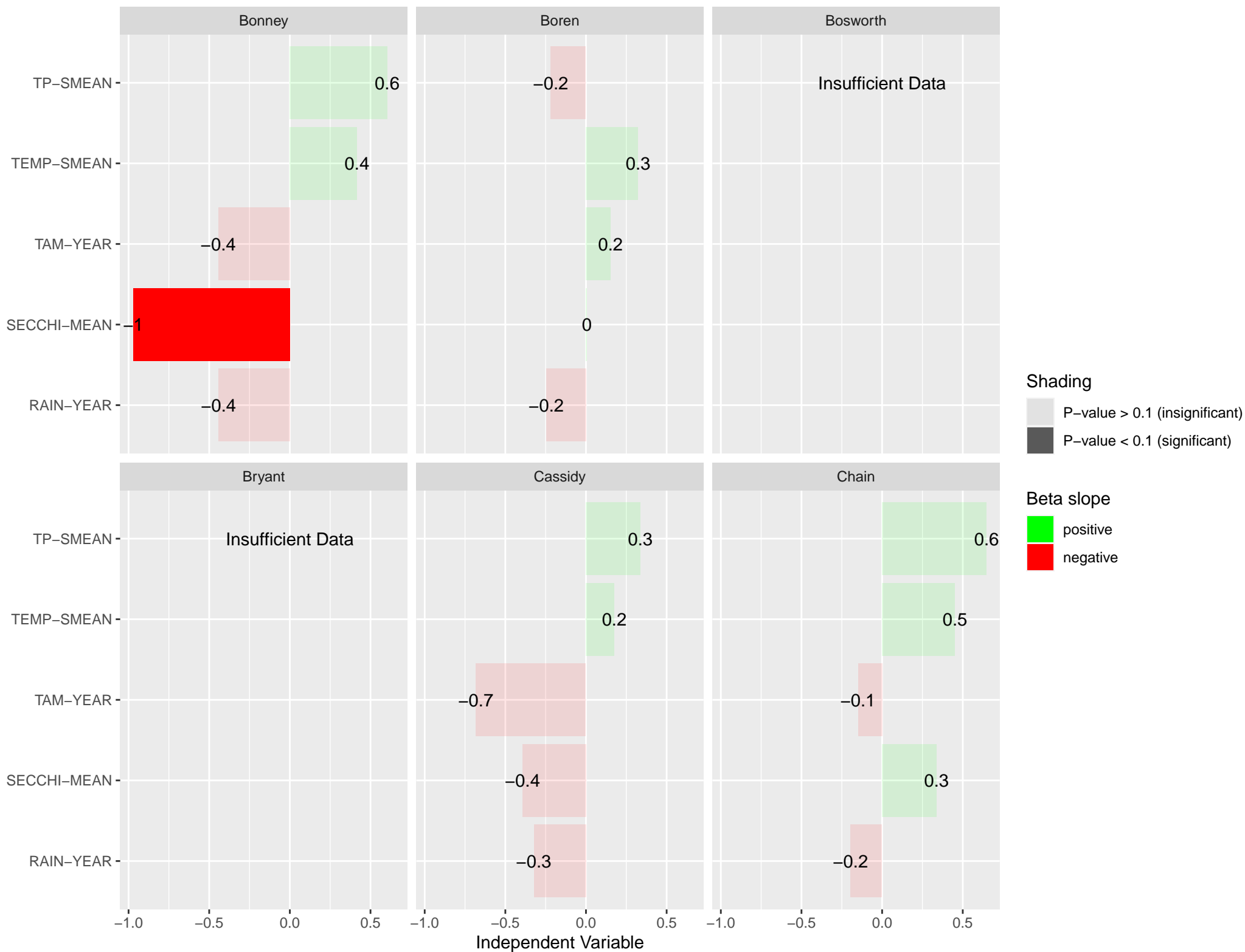
Multiple Regression Results for MC-MAX (Annual data)



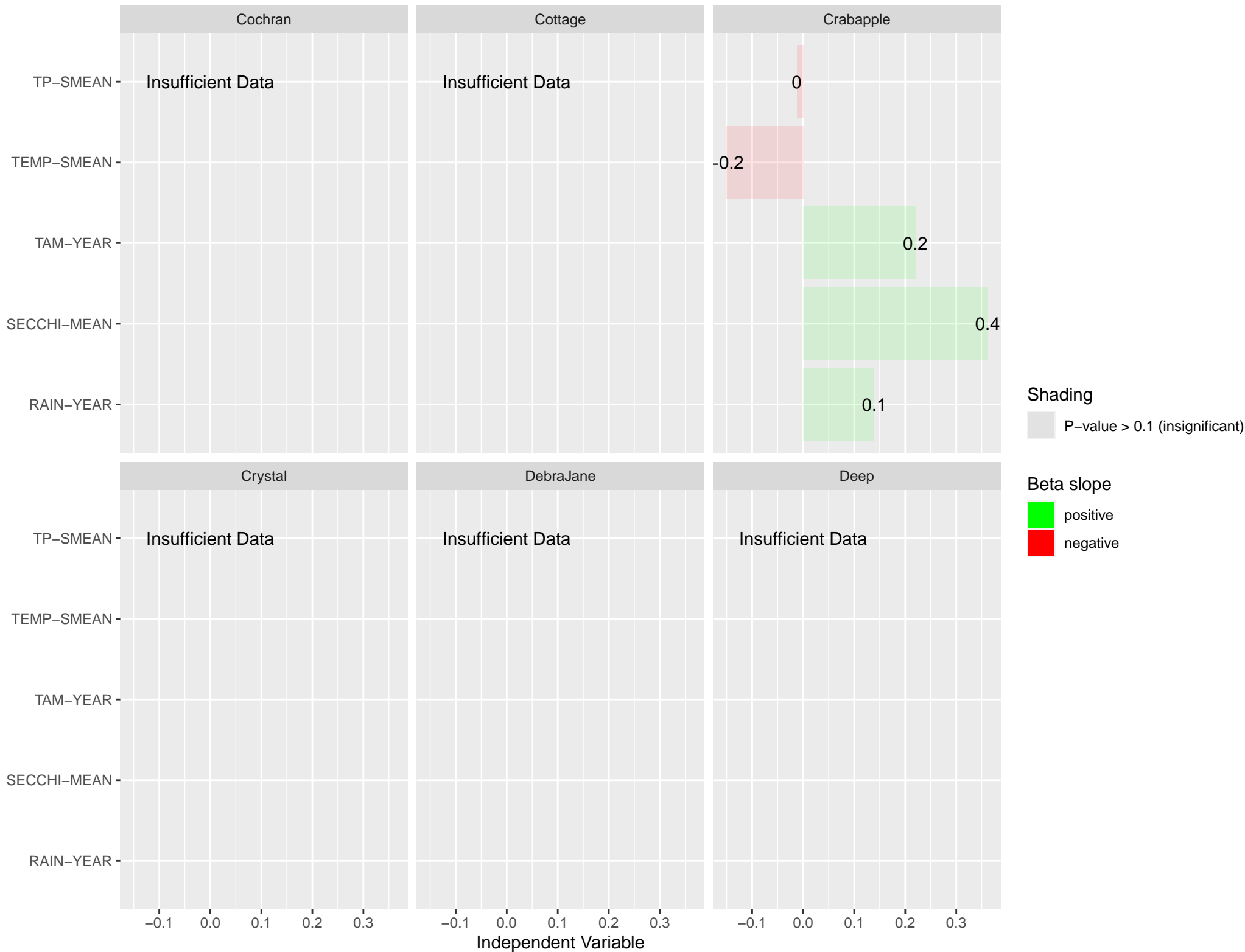
Multiple Regression Results for MC-MAX (Annual data)



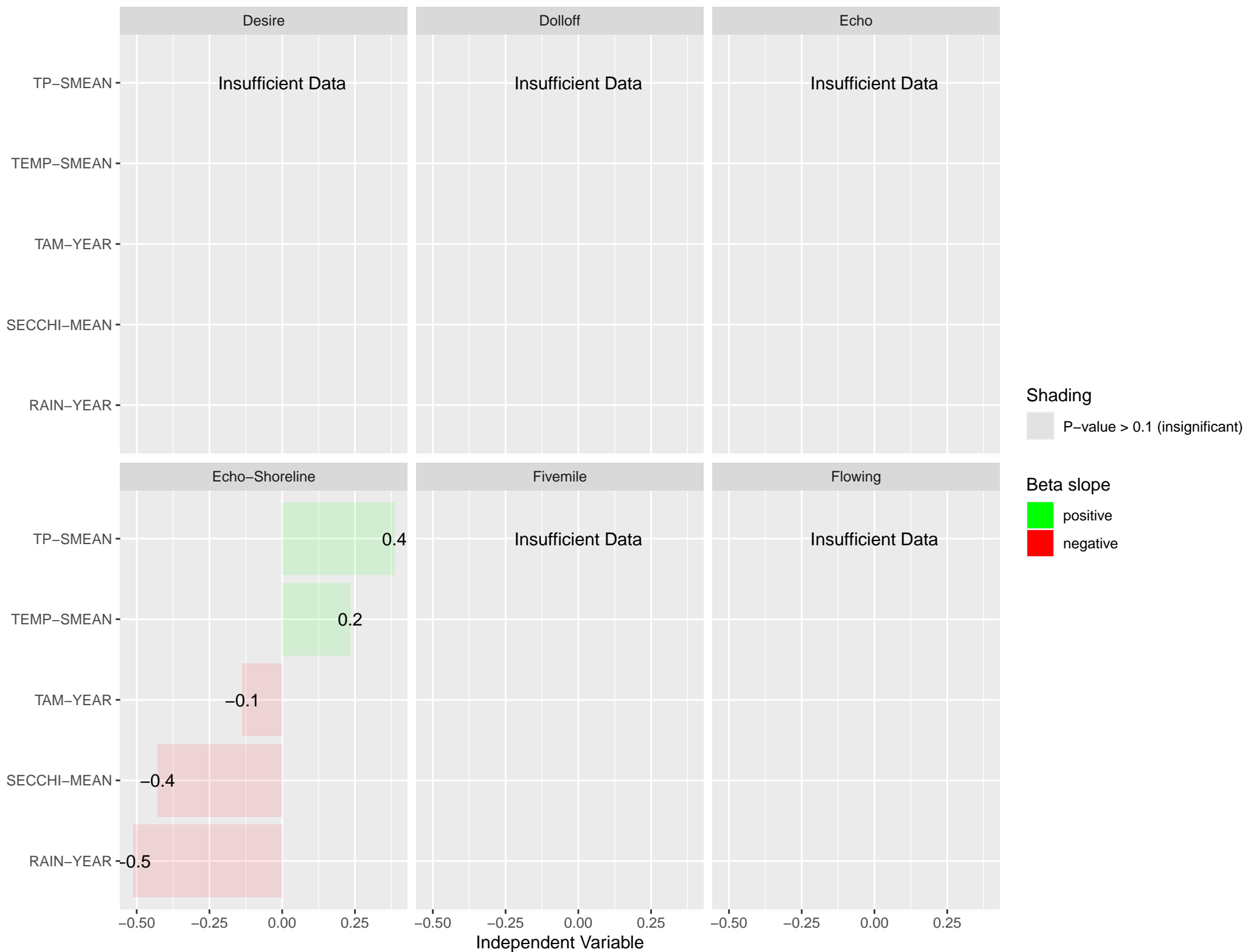
Multiple Regression Results for MC-MAX (Annual data)



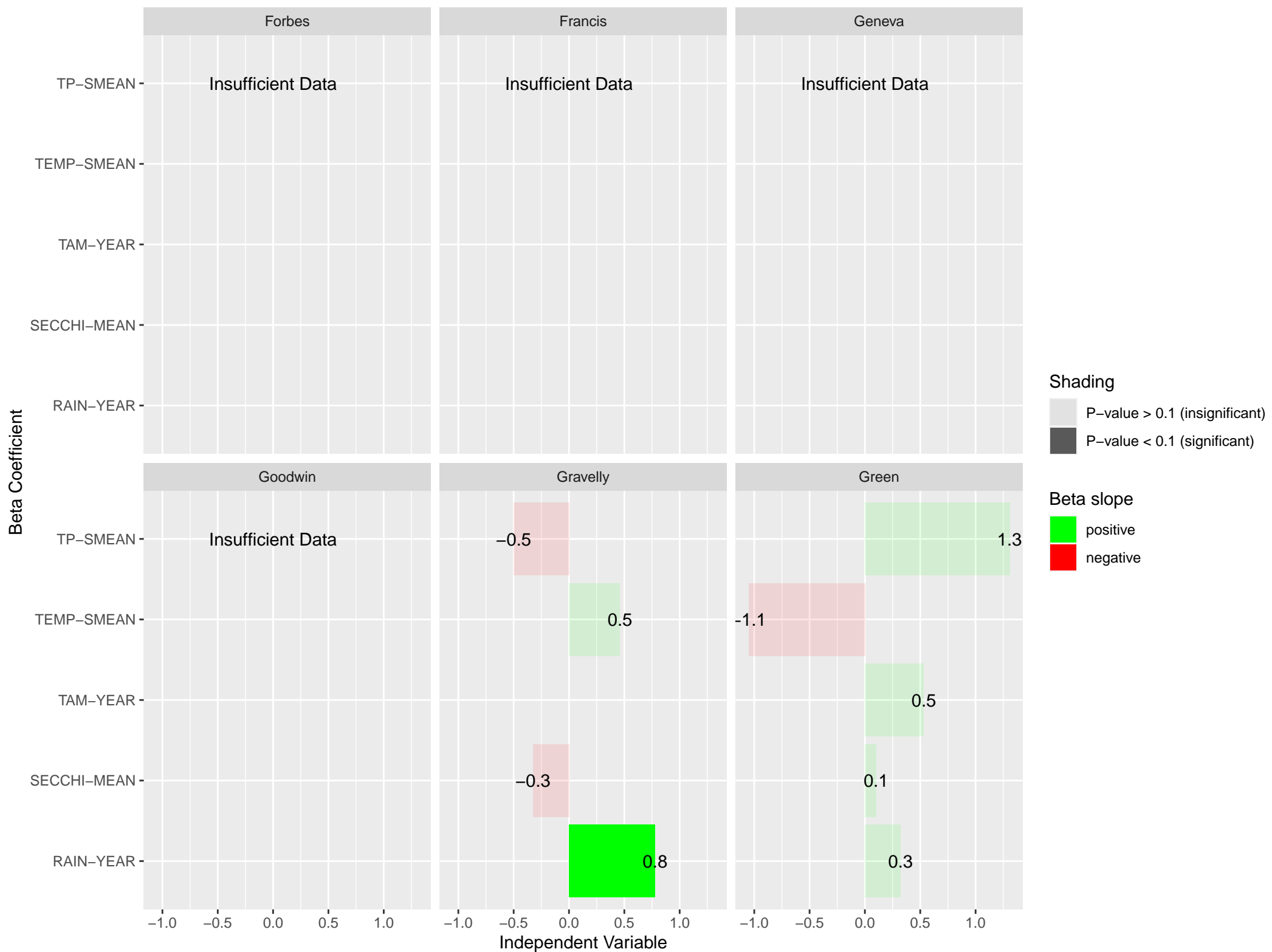
Multiple Regression Results for MC-MAX (Annual data)



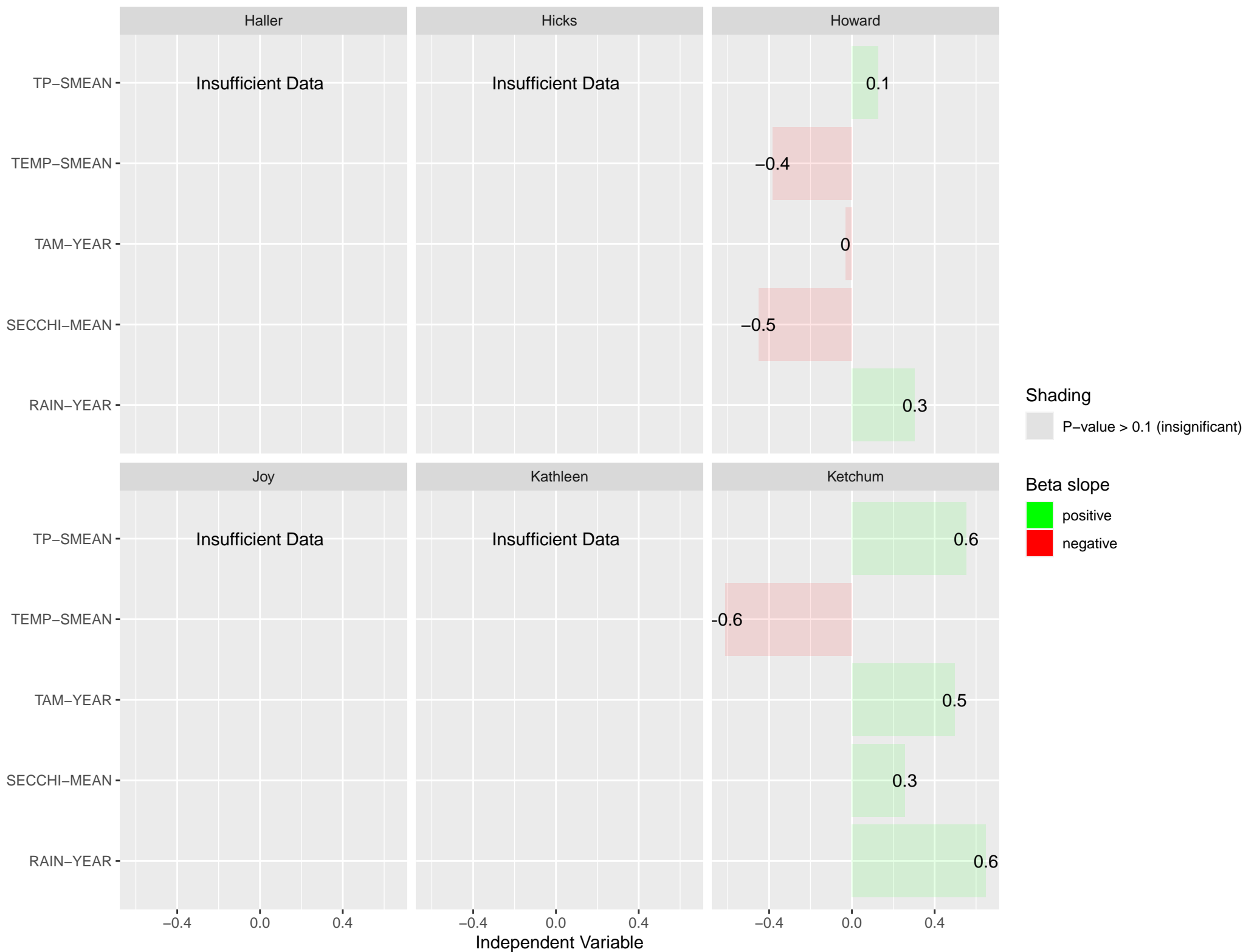
Multiple Regression Results for MC-MAX (Annual data)



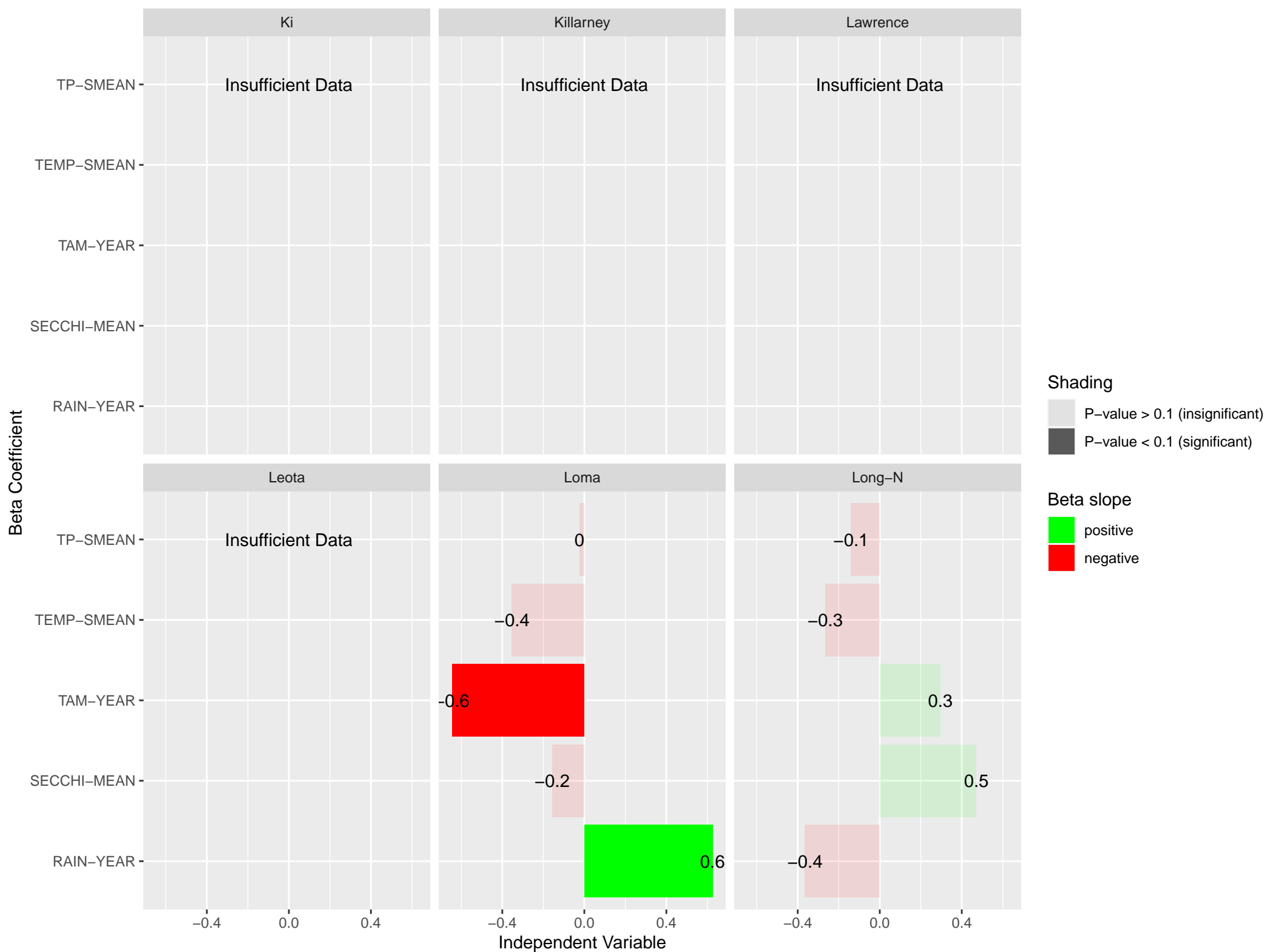
Multiple Regression Results for MC-MAX (Annual data)



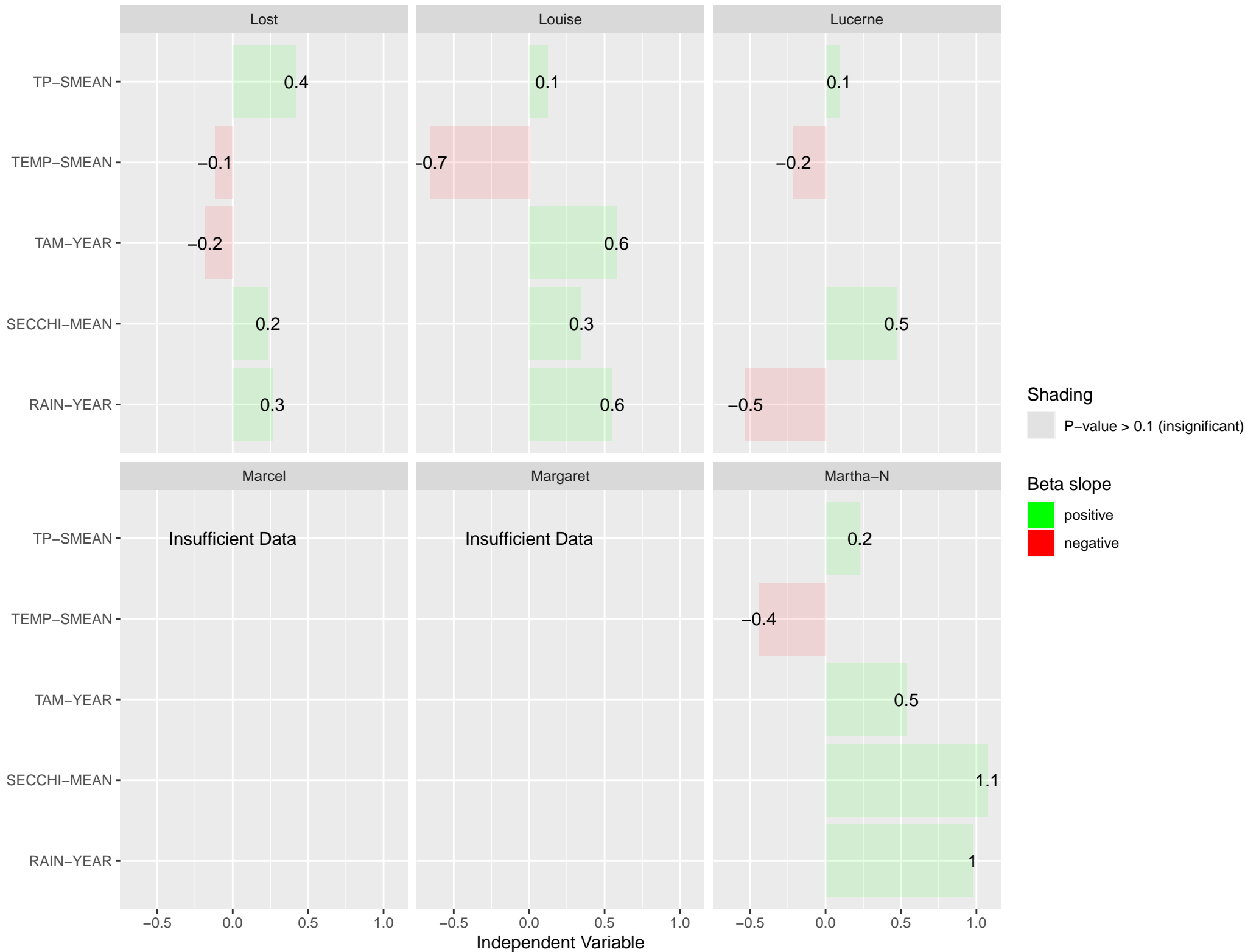
Multiple Regression Results for MC-MAX (Annual data)



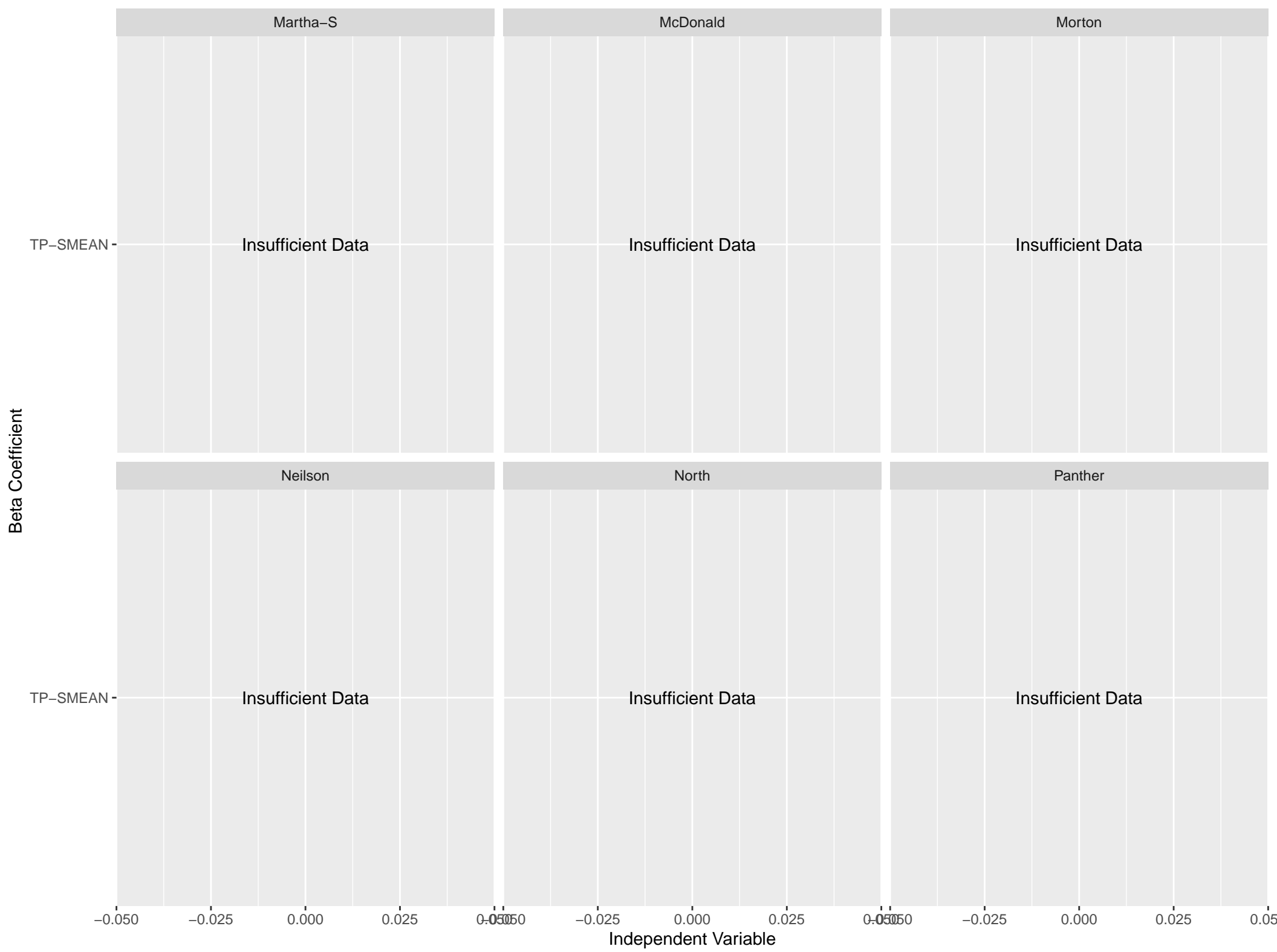
Multiple Regression Results for MC-MAX (Annual data)



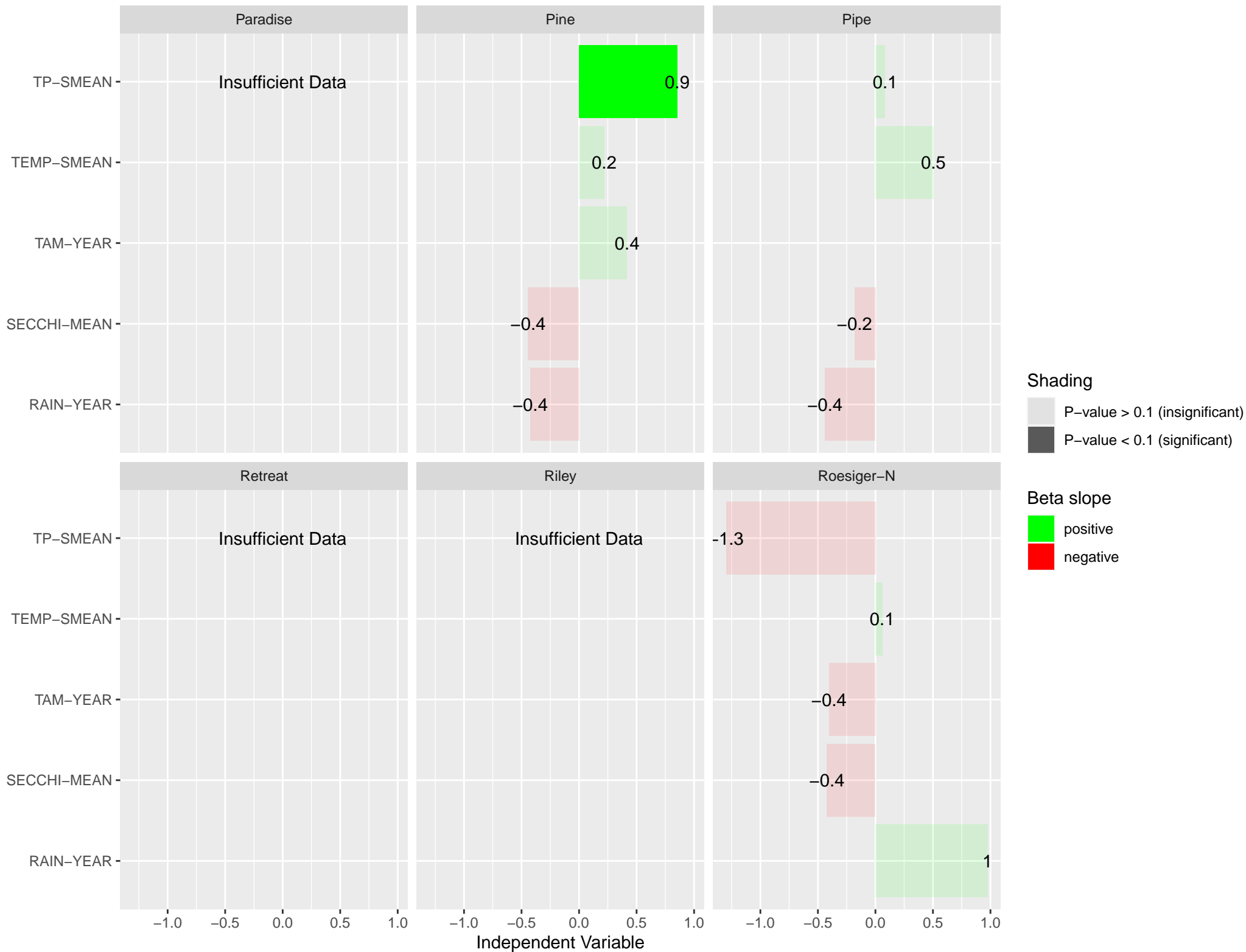
Multiple Regression Results for MC-MAX (Annual data)



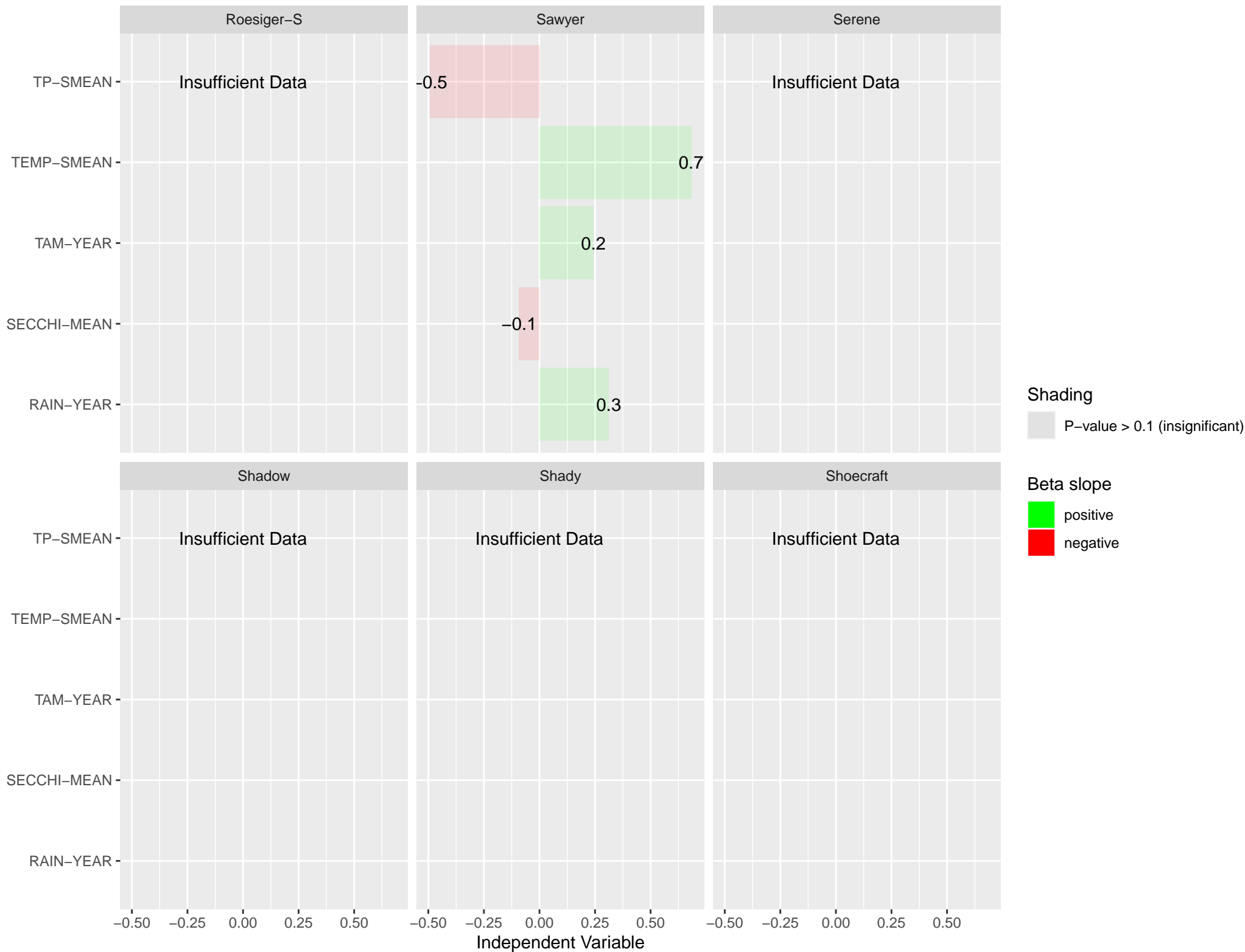
Multiple Regression Results for MC-MAX (Annual data)



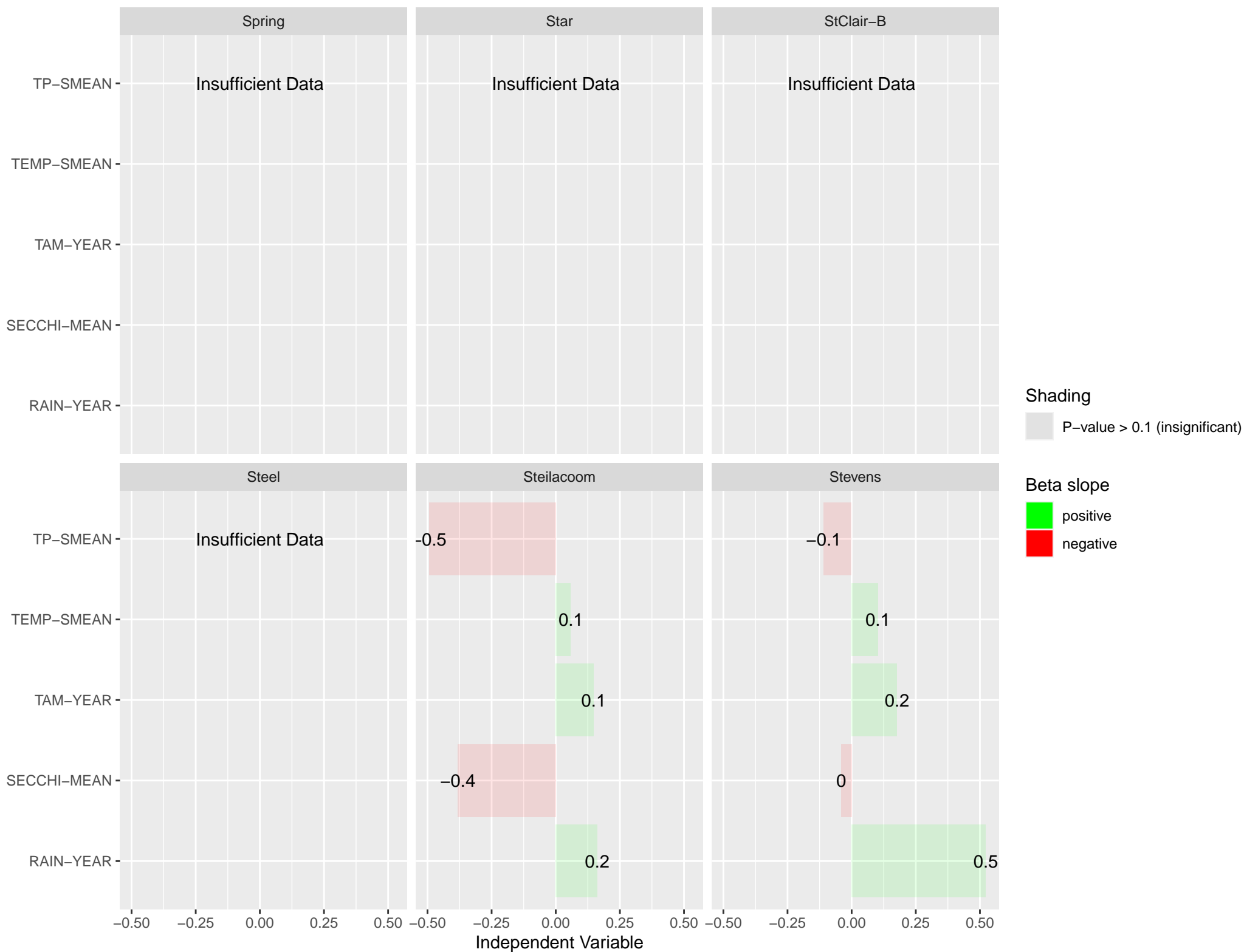
Multiple Regression Results for MC-MAX (Annual data)



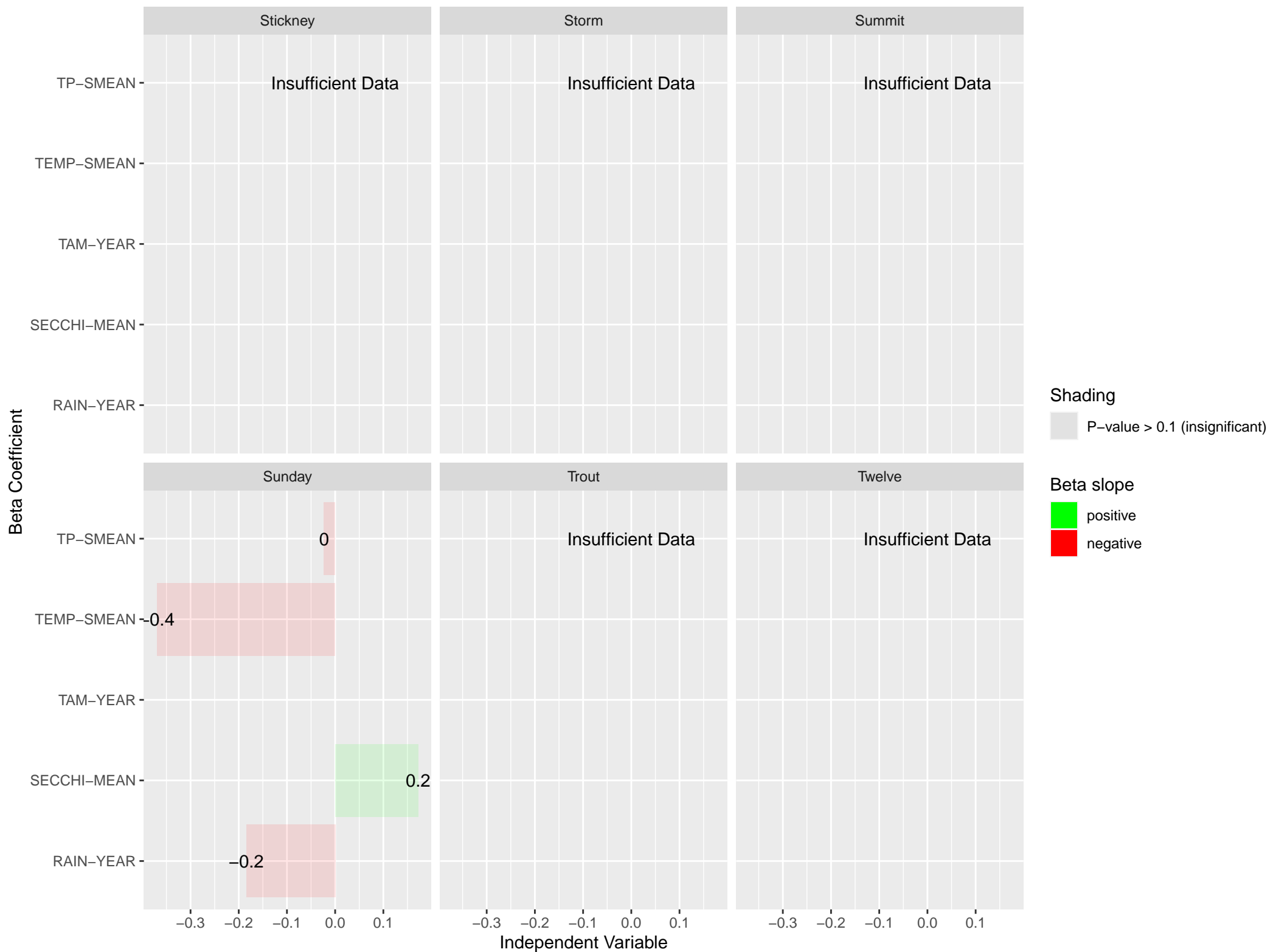
Multiple Regression Results for MC-MAX (Annual data)



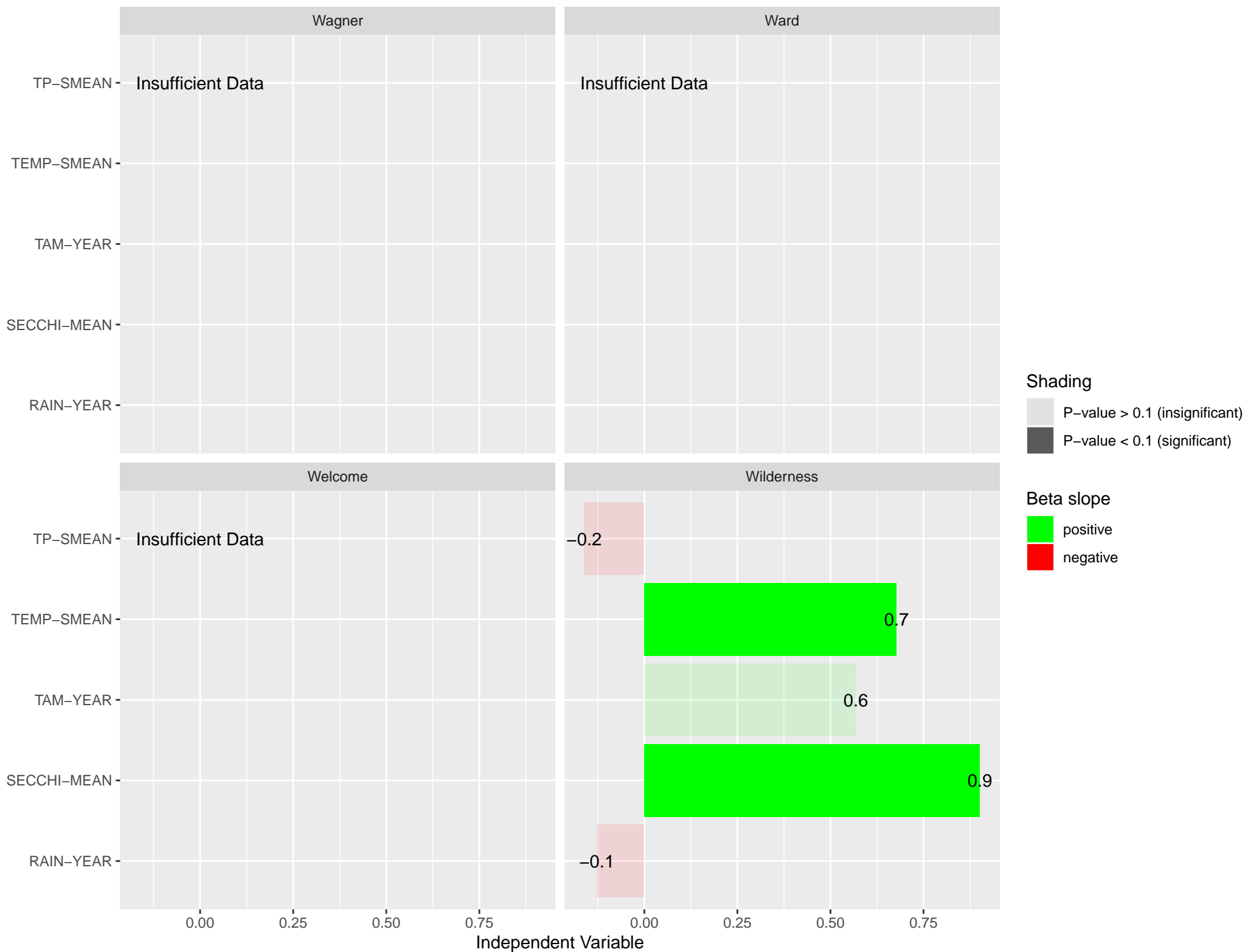
Multiple Regression Results for MC-MAX (Annual data)



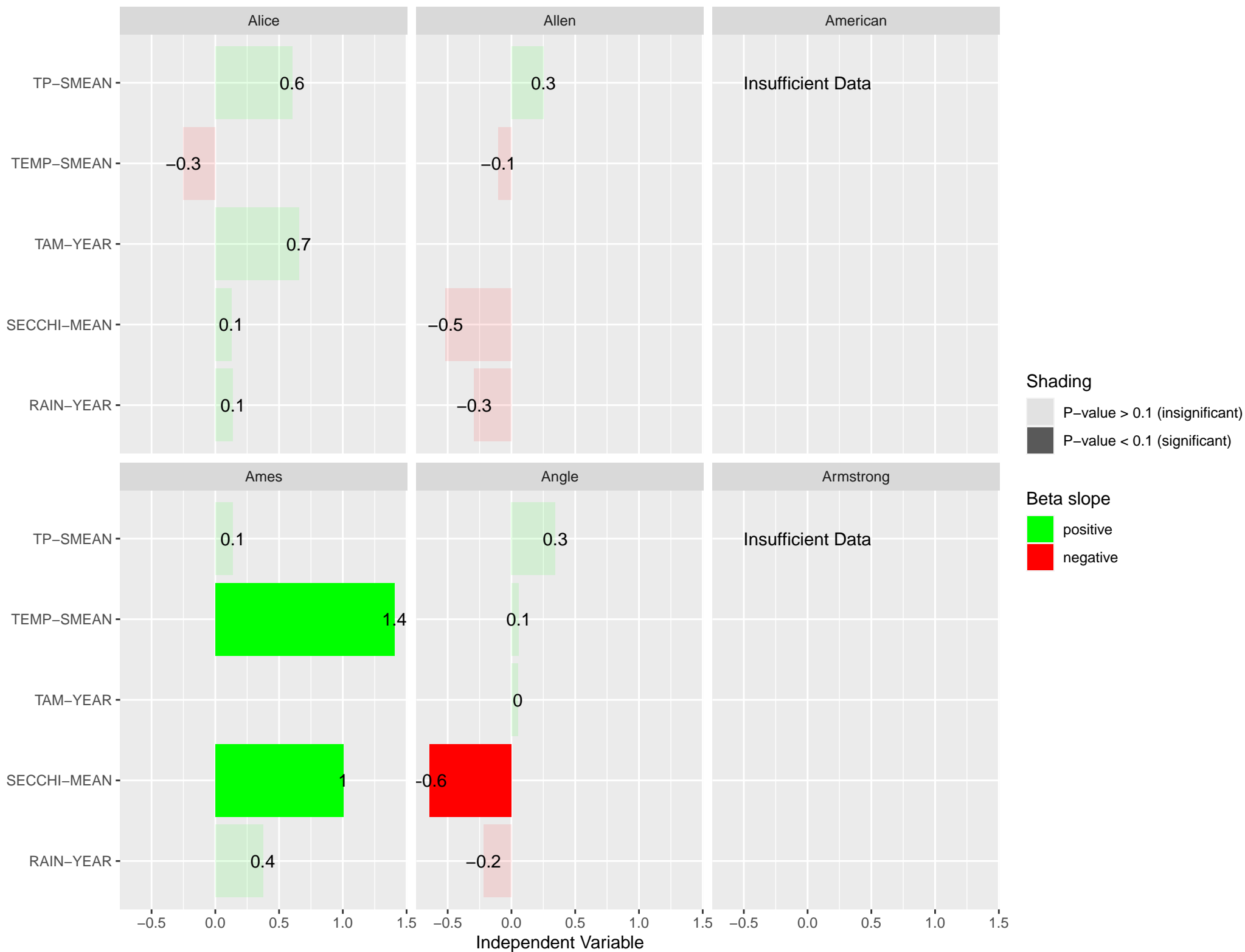
Multiple Regression Results for MC-MAX (Annual data)



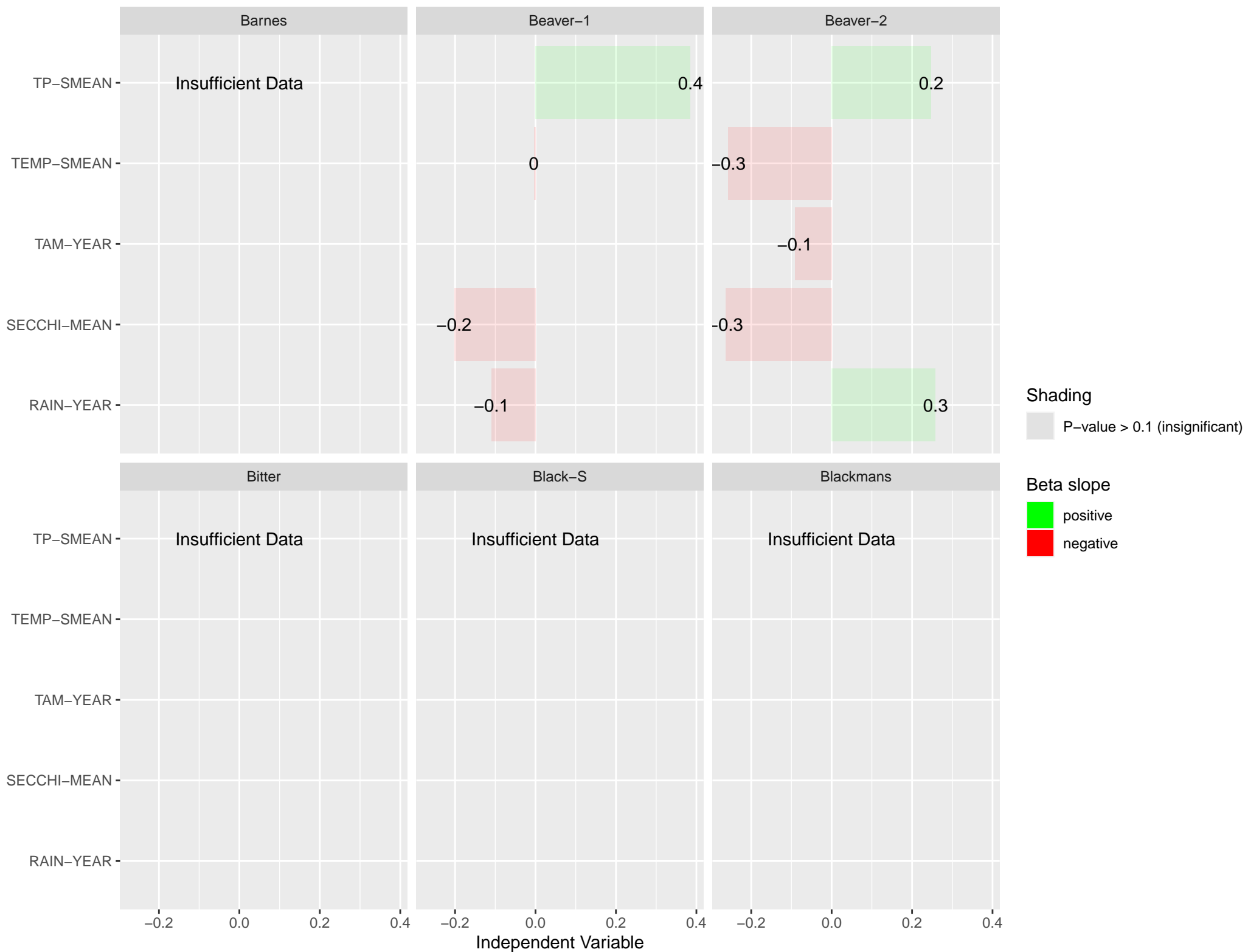
Multiple Regression Results for MC-MAX (Annual data)



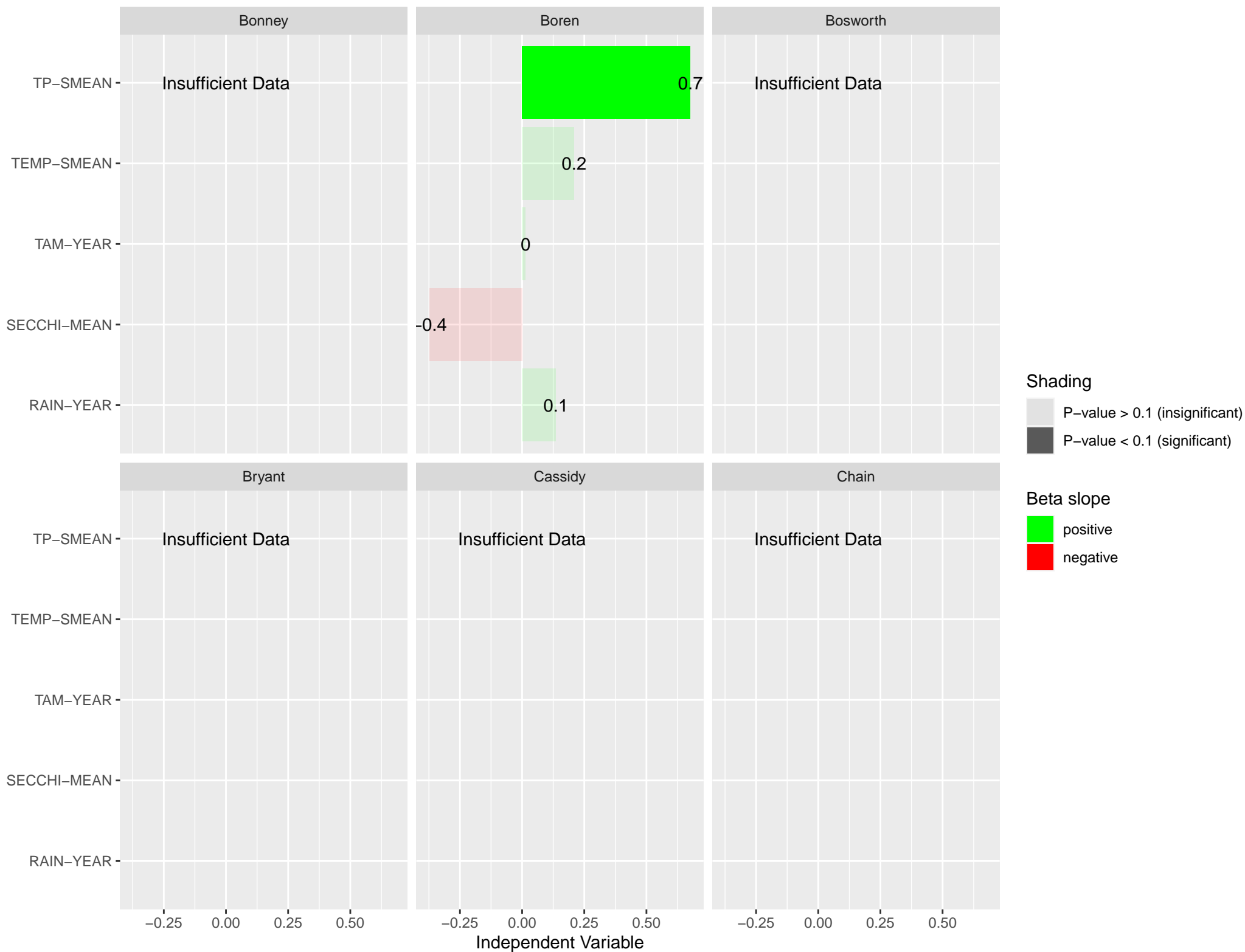
Multiple Regression Results for CHLA-SMEAN (Spring data)



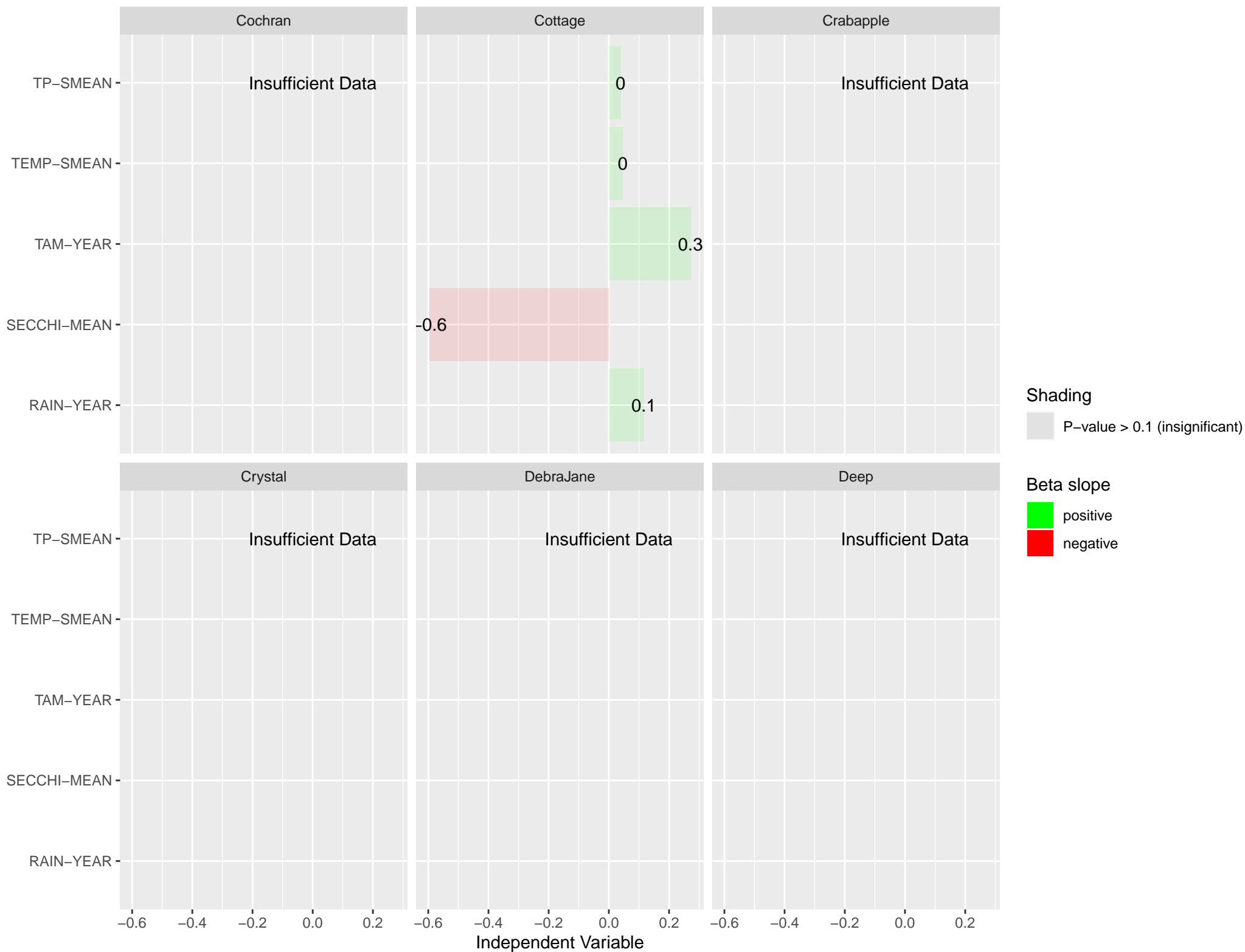
Multiple Regression Results for CHLA-SMEAN (Spring data)



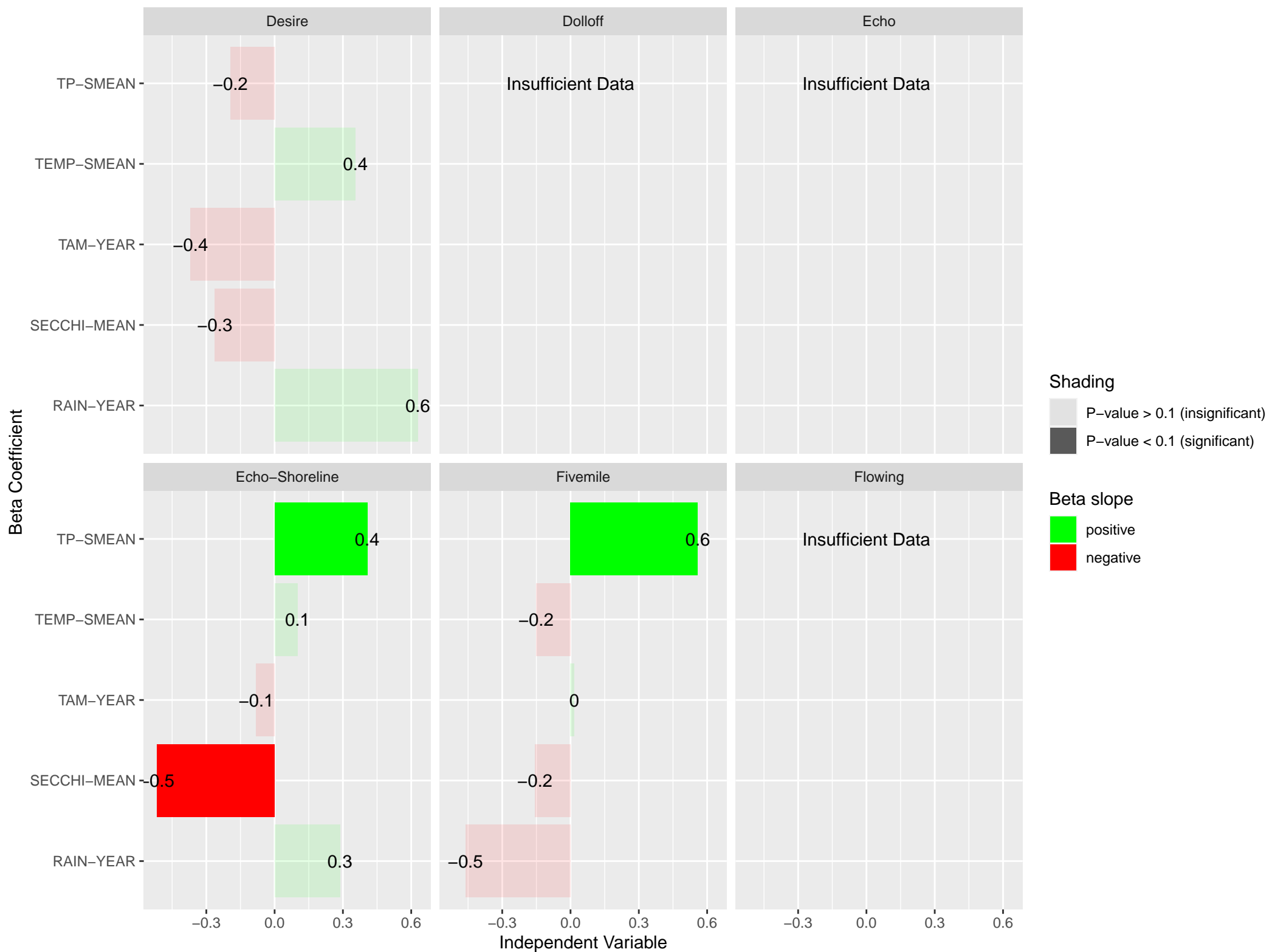
Multiple Regression Results for CHLA-SMEAN (Spring data)



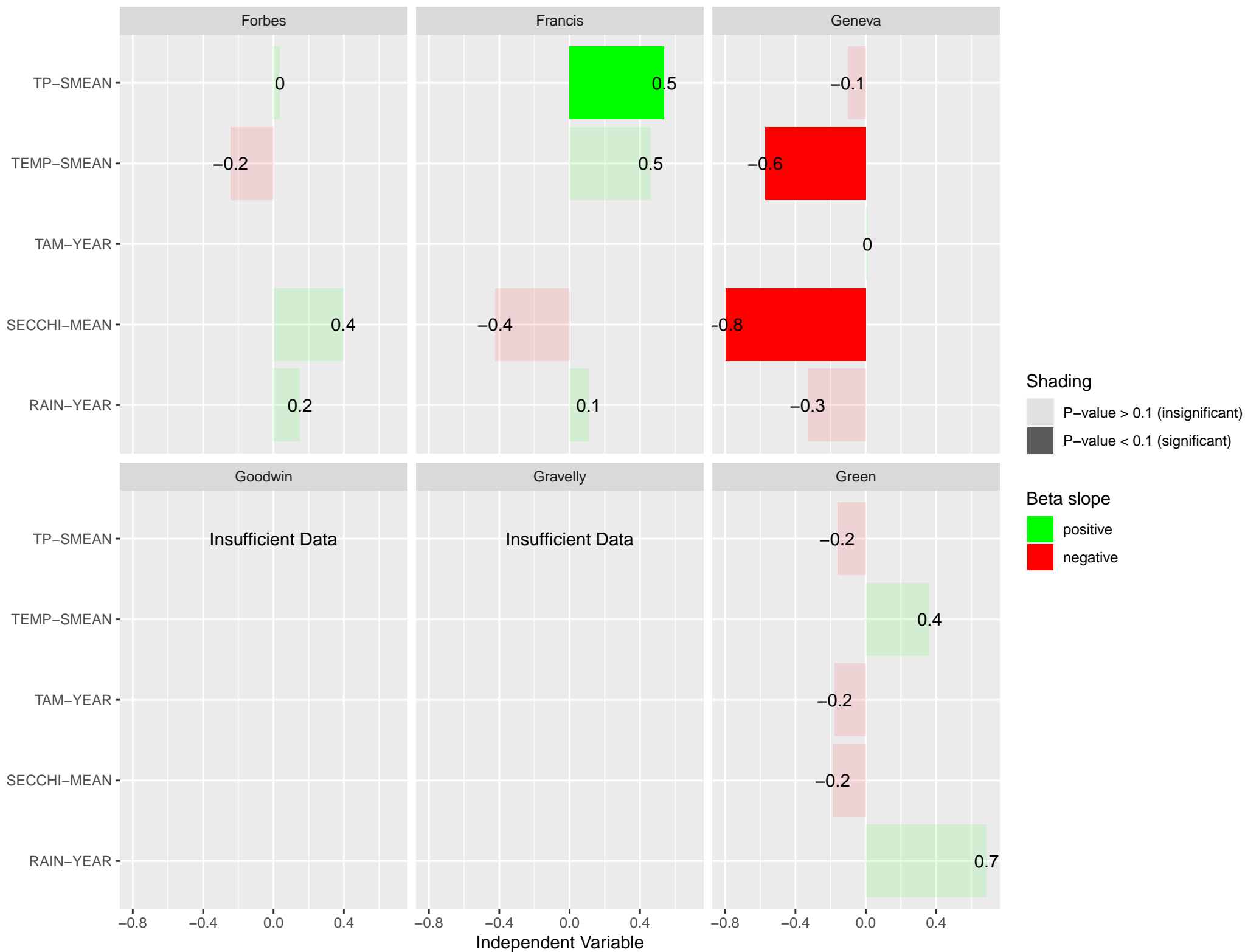
Multiple Regression Results for CHLA-SMEAN (Spring data)



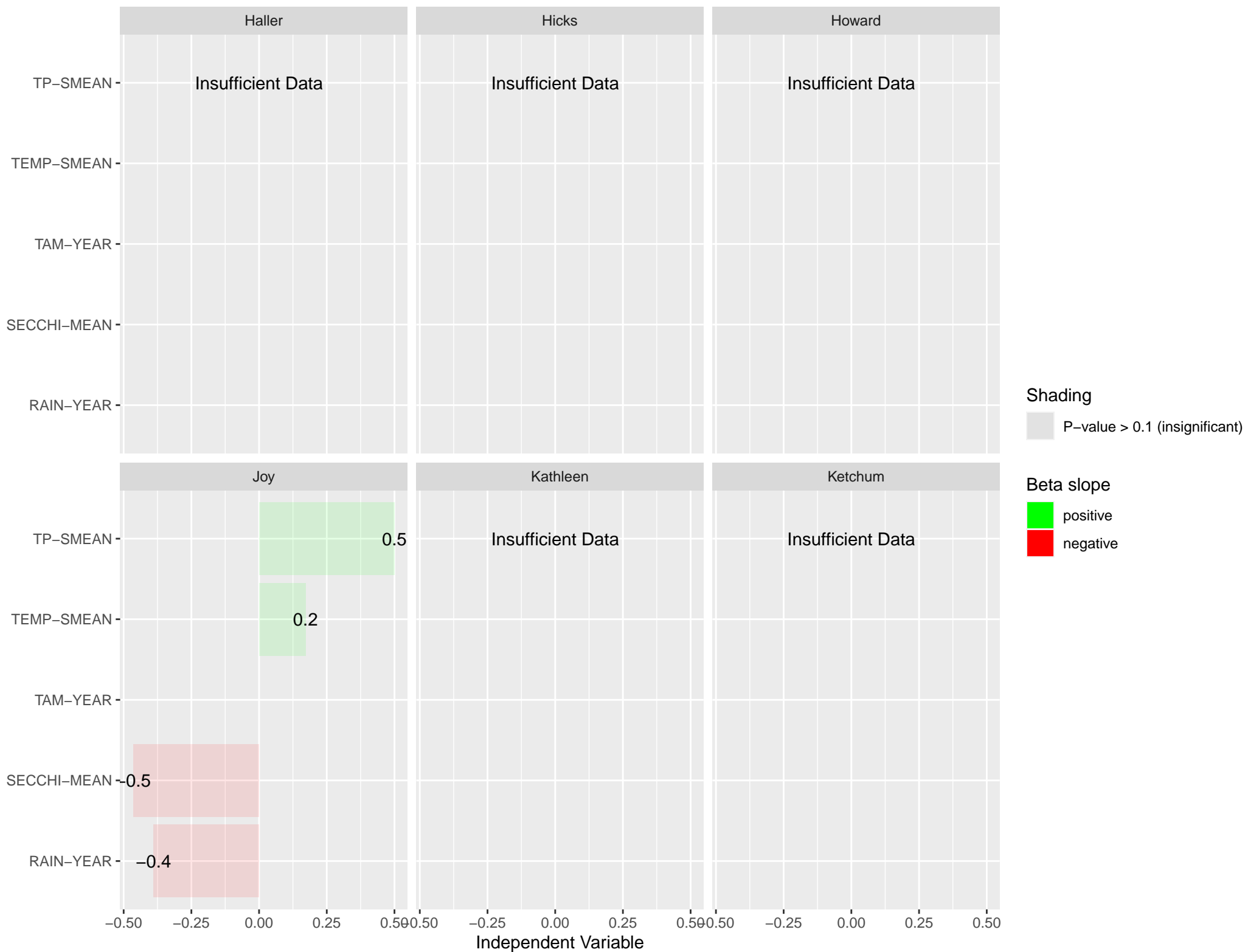
Multiple Regression Results for CHLA-SMEAN (Spring data)



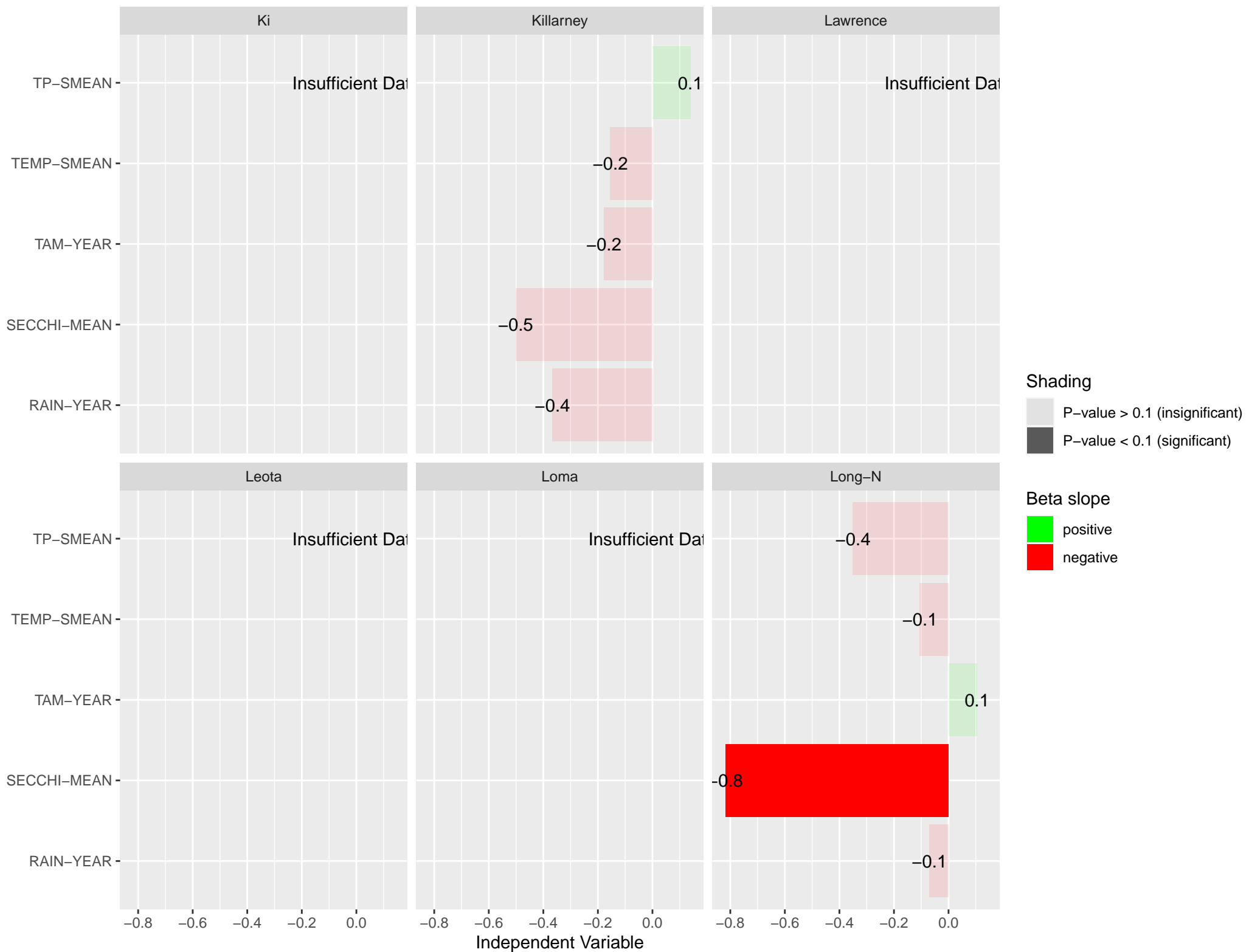
Multiple Regression Results for CHLA-SMEAN (Spring data)



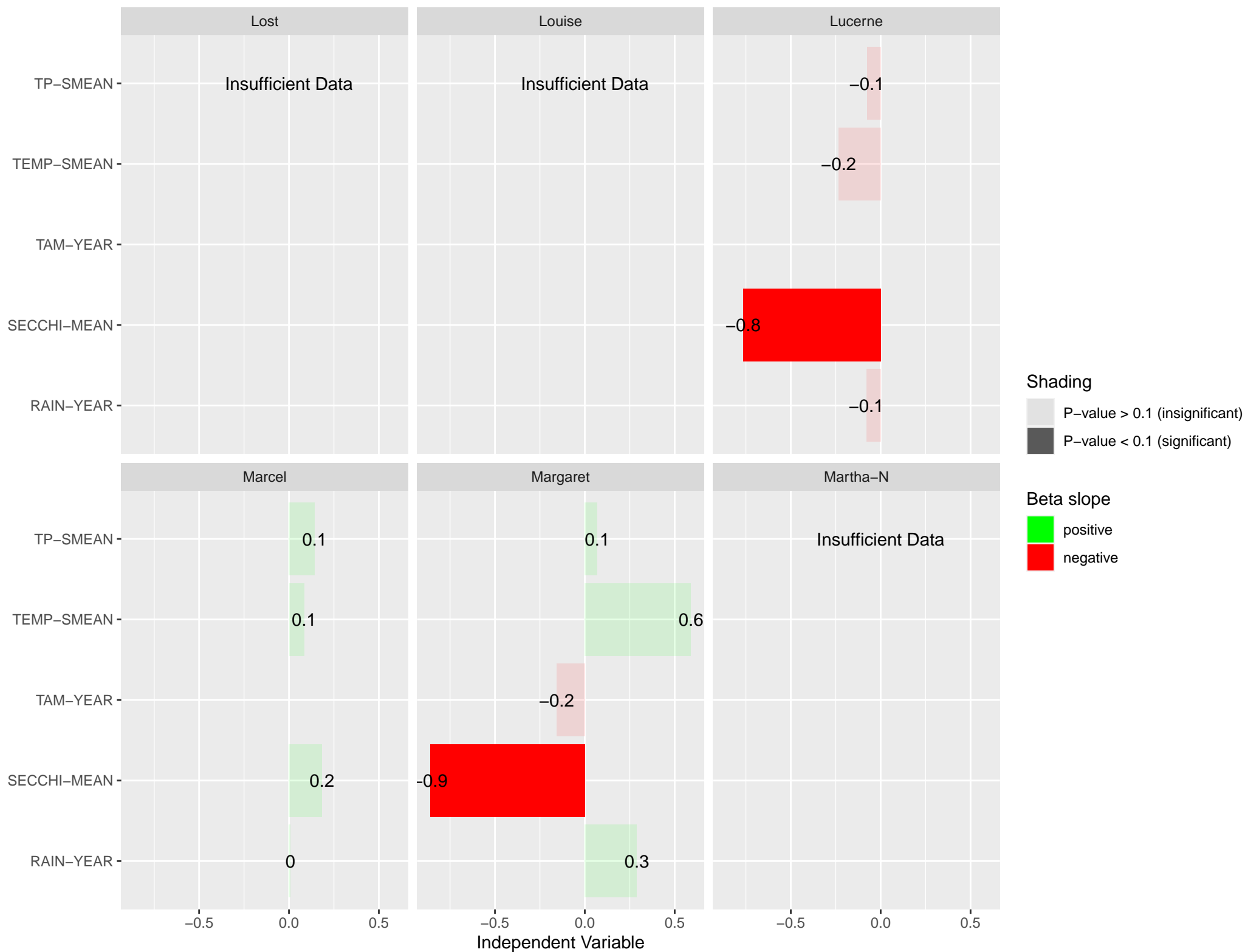
Multiple Regression Results for CHLA-SMEAN (Spring data)



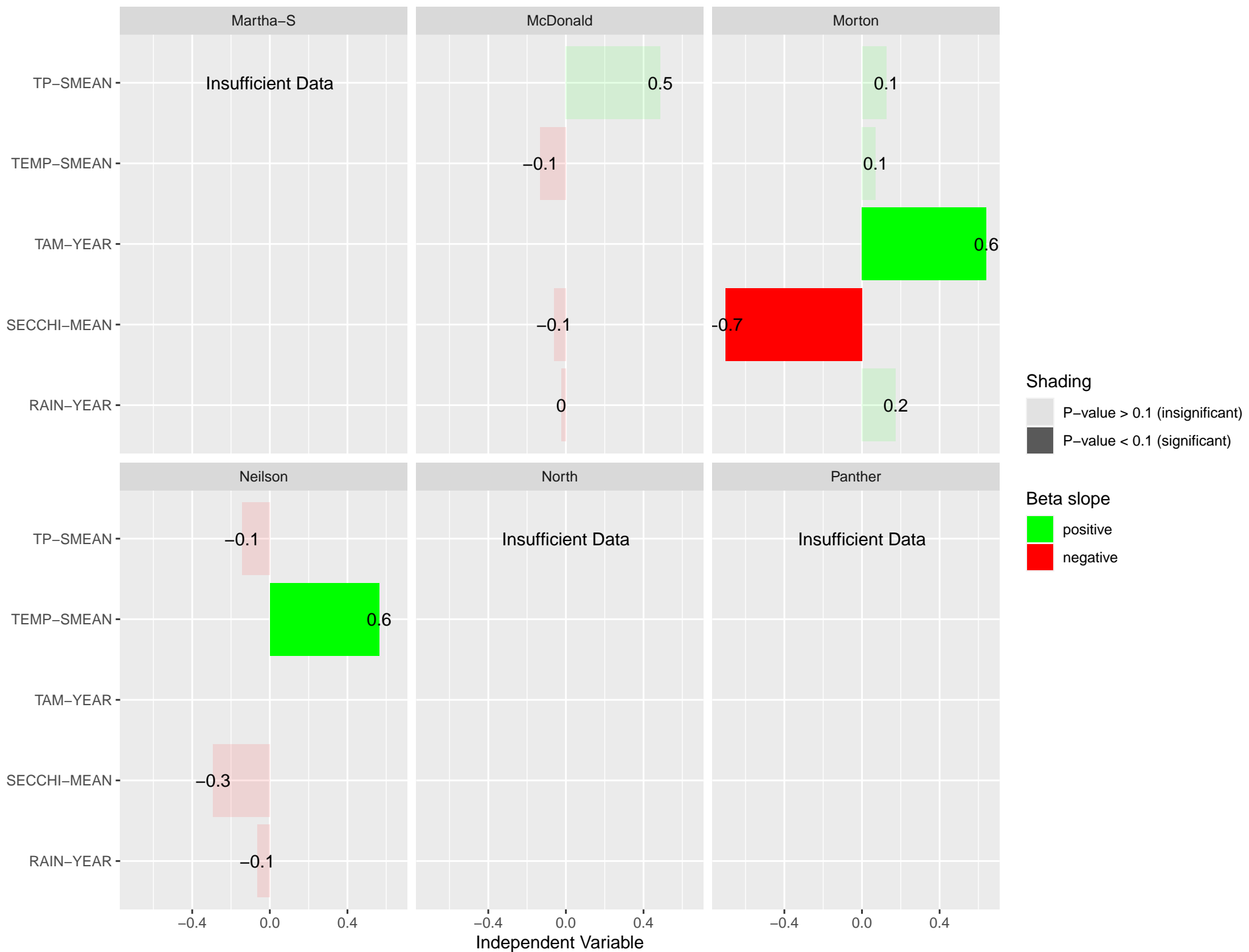
Multiple Regression Results for CHLA-SMEAN (Spring data)



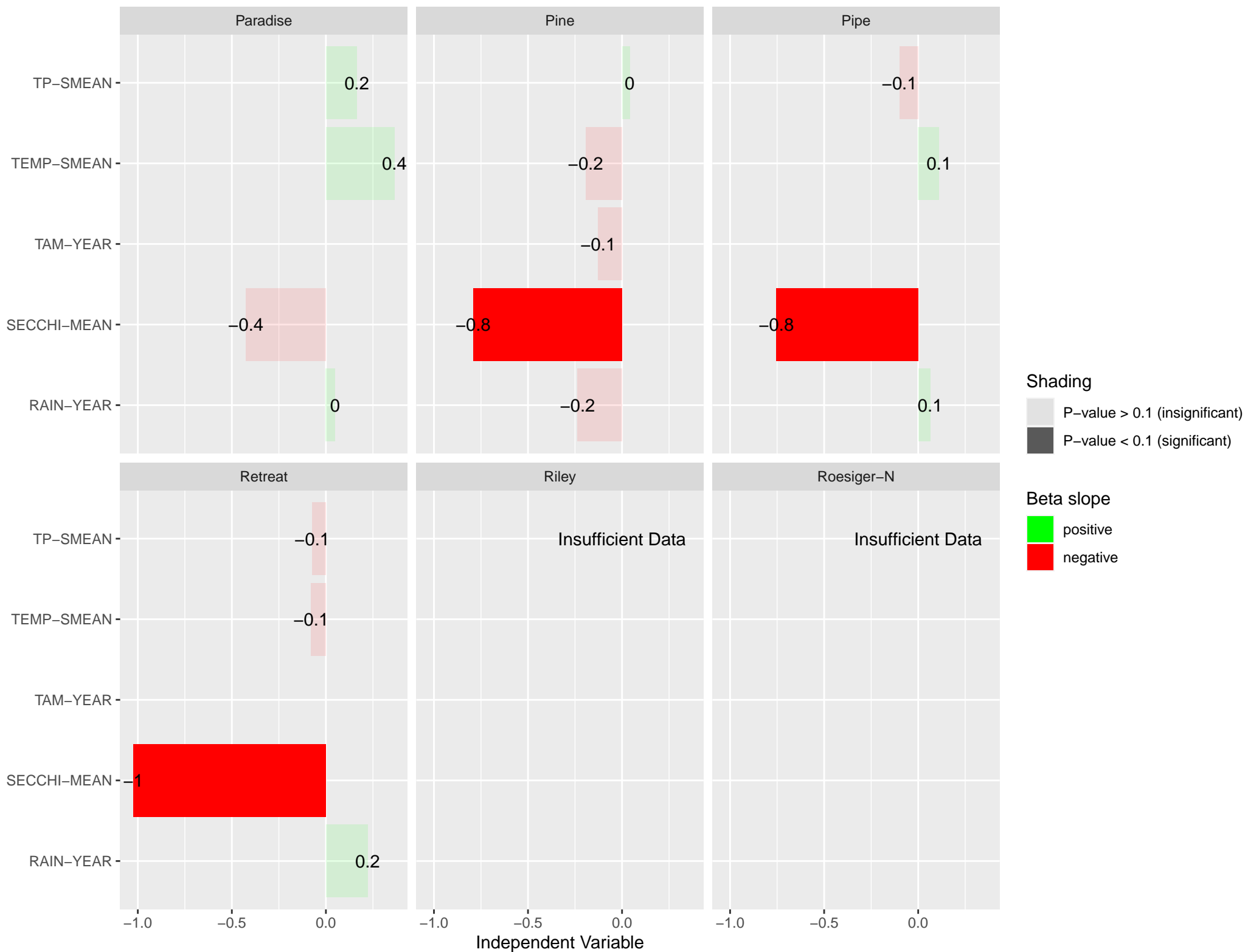
Multiple Regression Results for CHLA-SMEAN (Spring data)



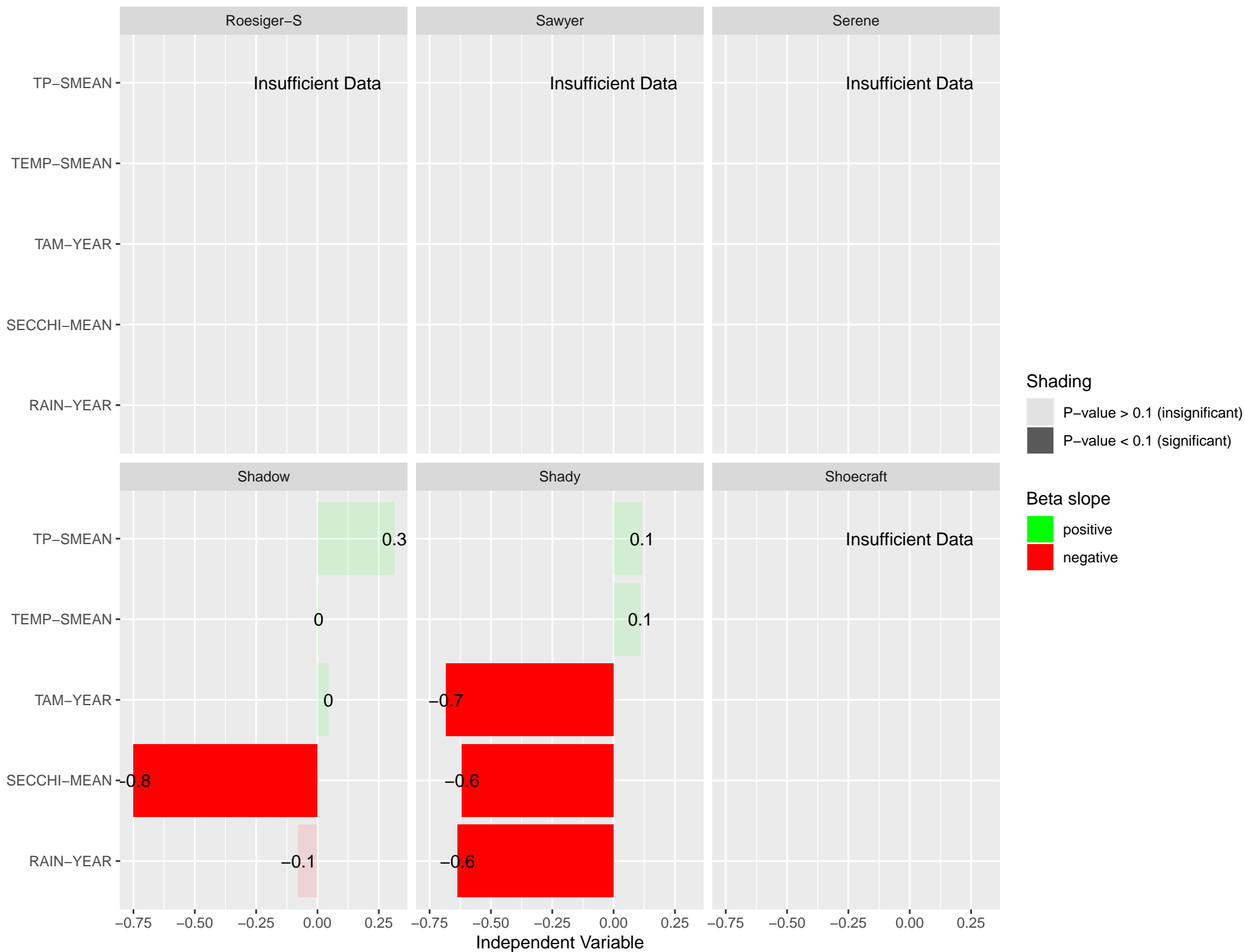
Multiple Regression Results for CHLA-SMEAN (Spring data)



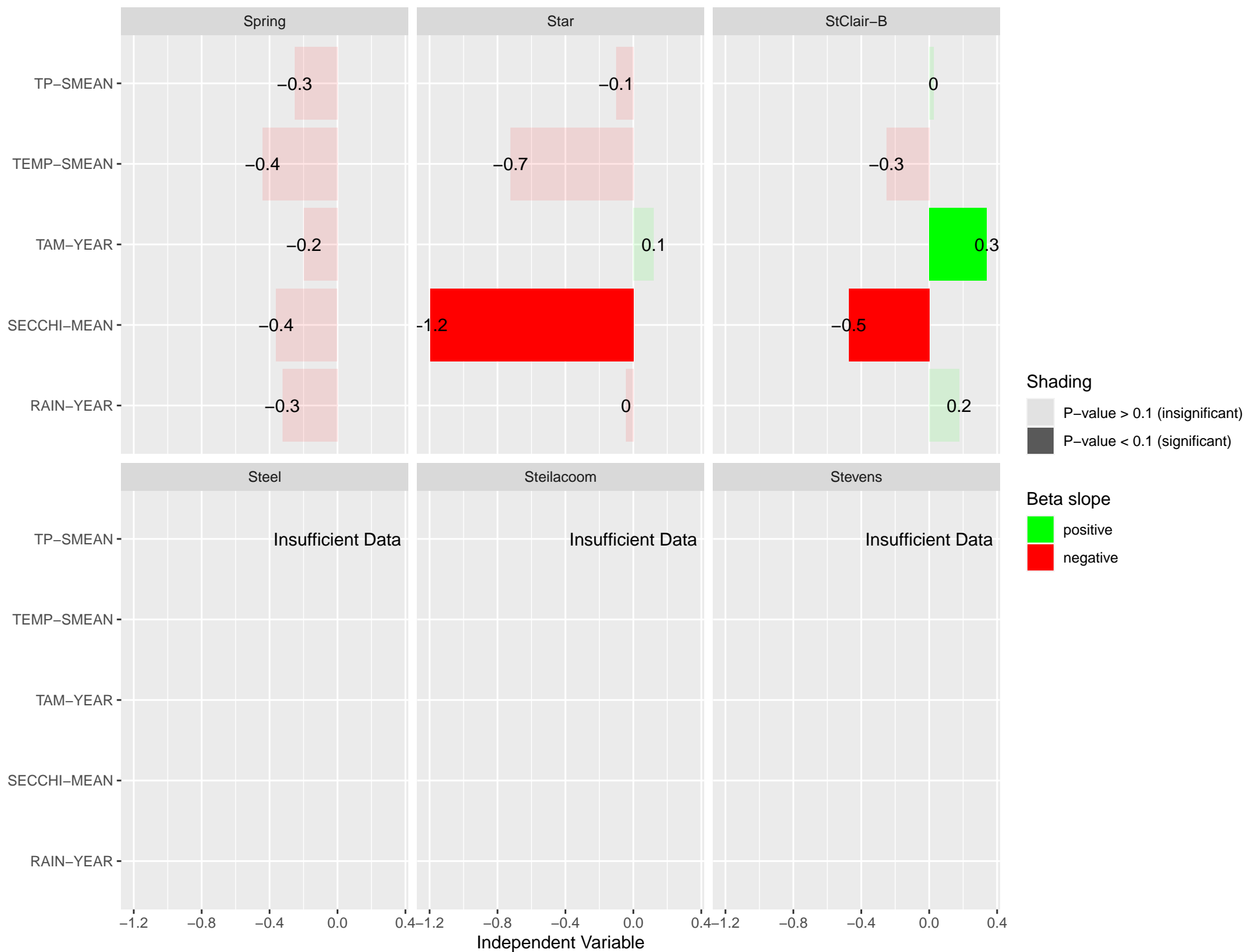
Multiple Regression Results for CHLA-SMEAN (Spring data)



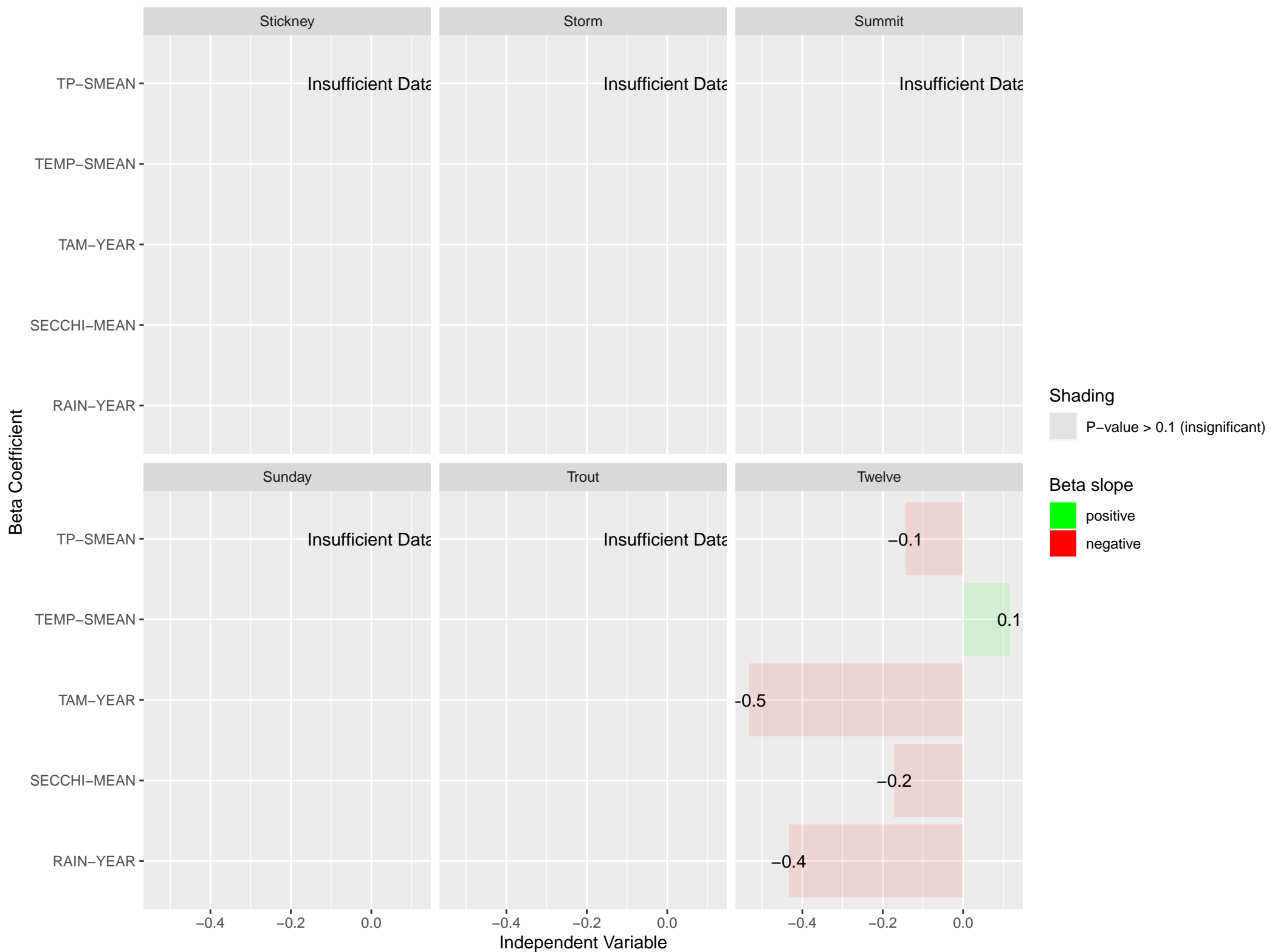
Multiple Regression Results for CHLA-SMEAN (Spring data)



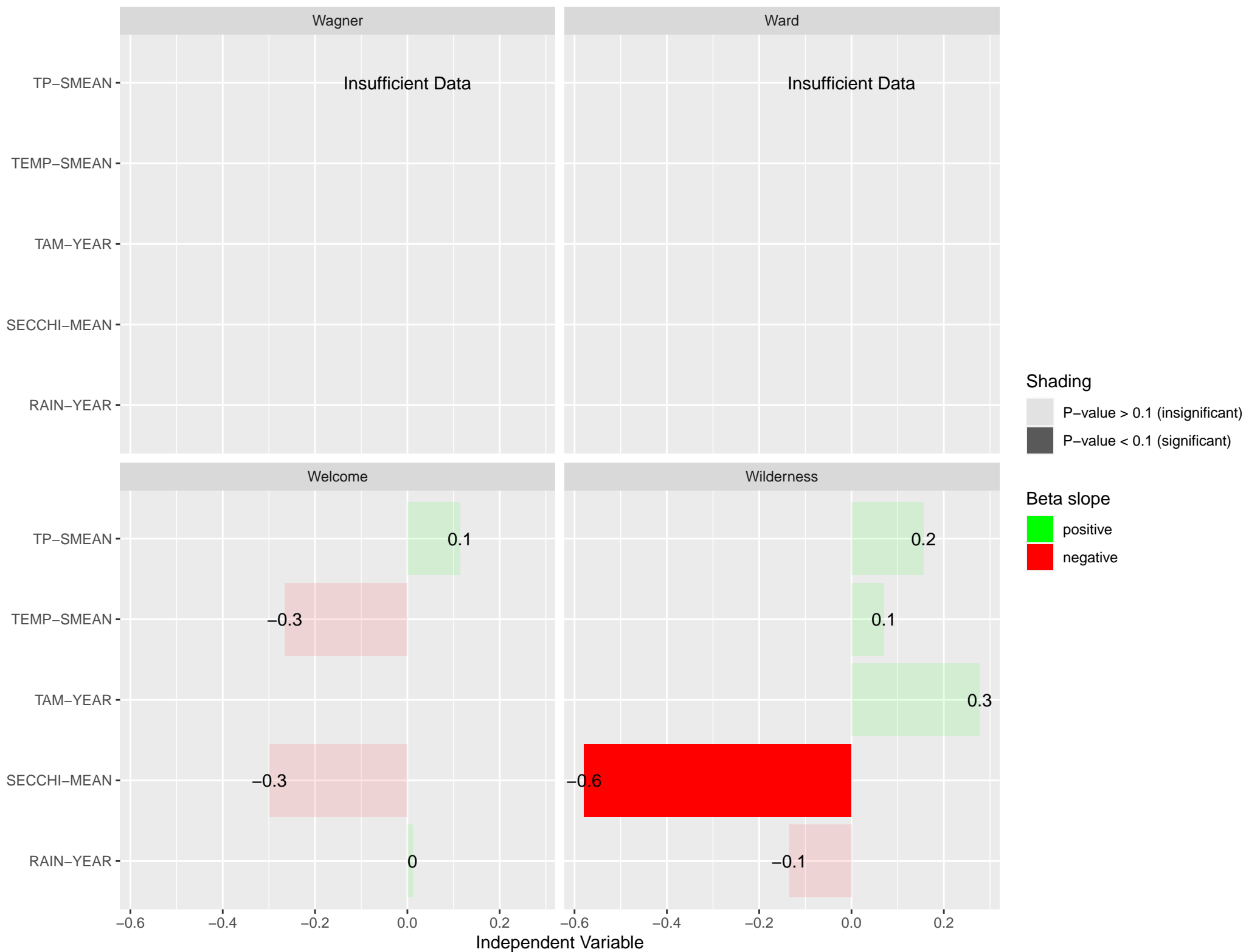
Multiple Regression Results for CHLA-SMEAN (Spring data)



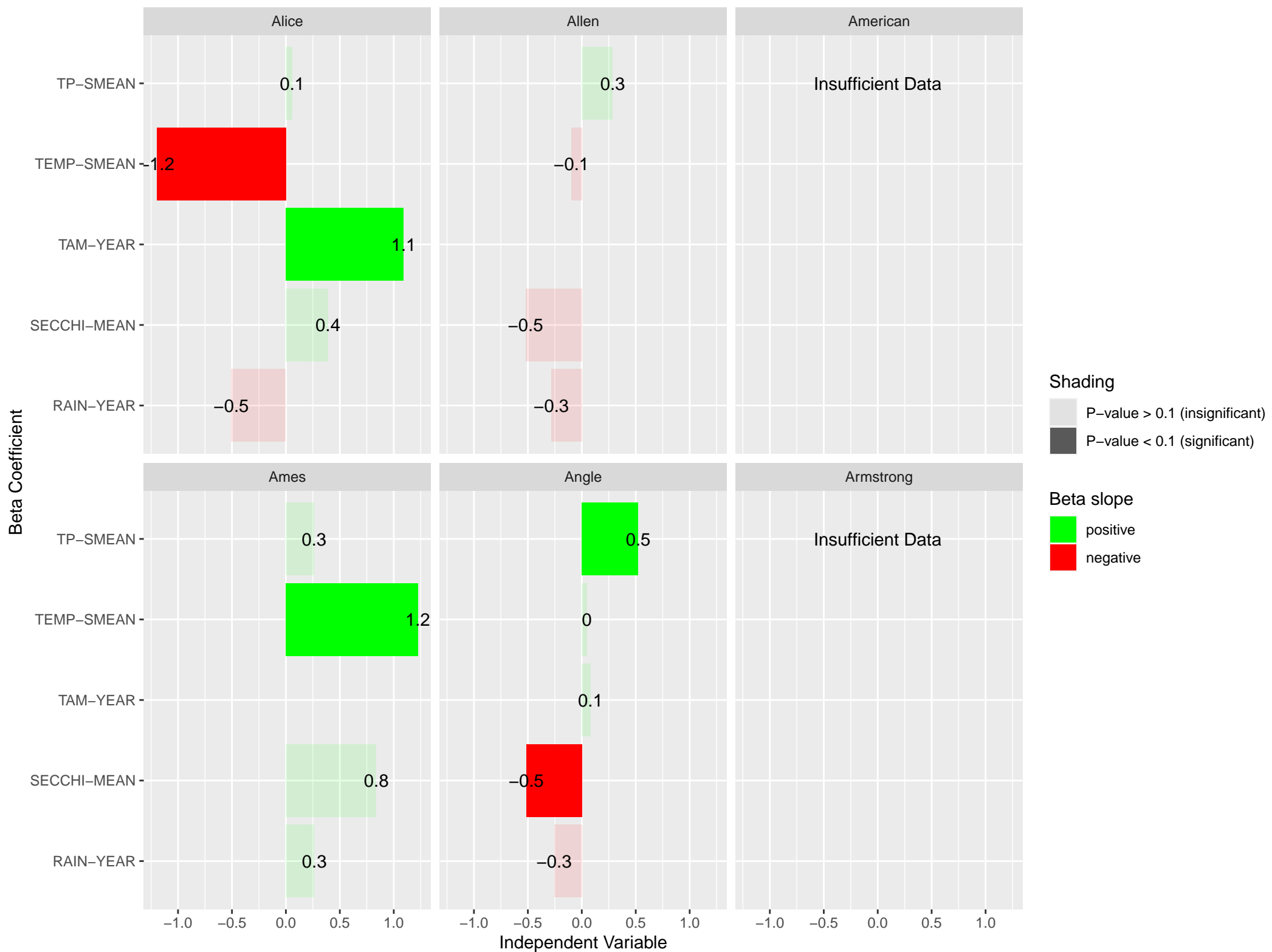
Multiple Regression Results for CHLA-SMEAN (Spring data)



Multiple Regression Results for CHLA-SMEAN (Spring data)



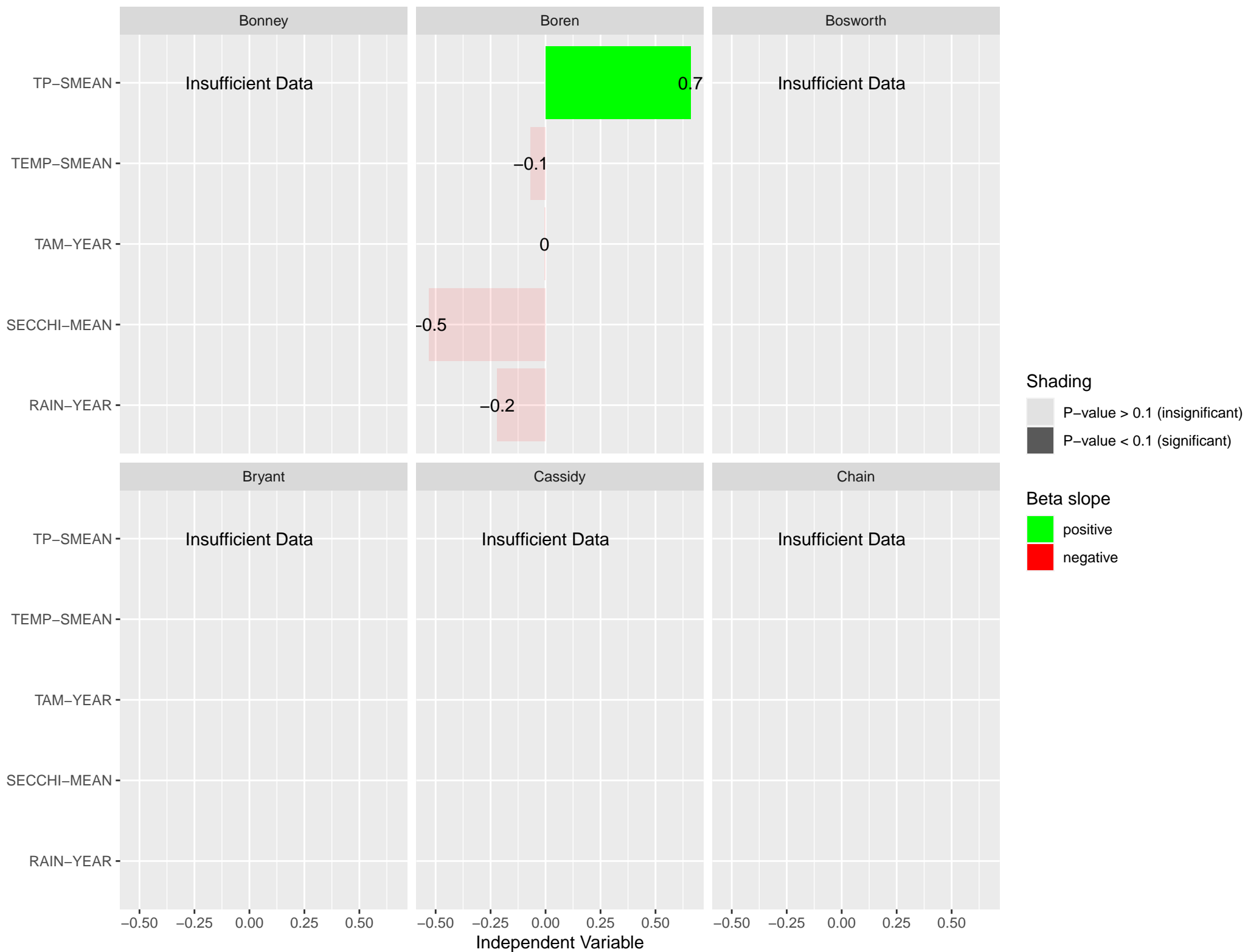
Multiple Regression Results for CHLA-SMAX (Spring data)



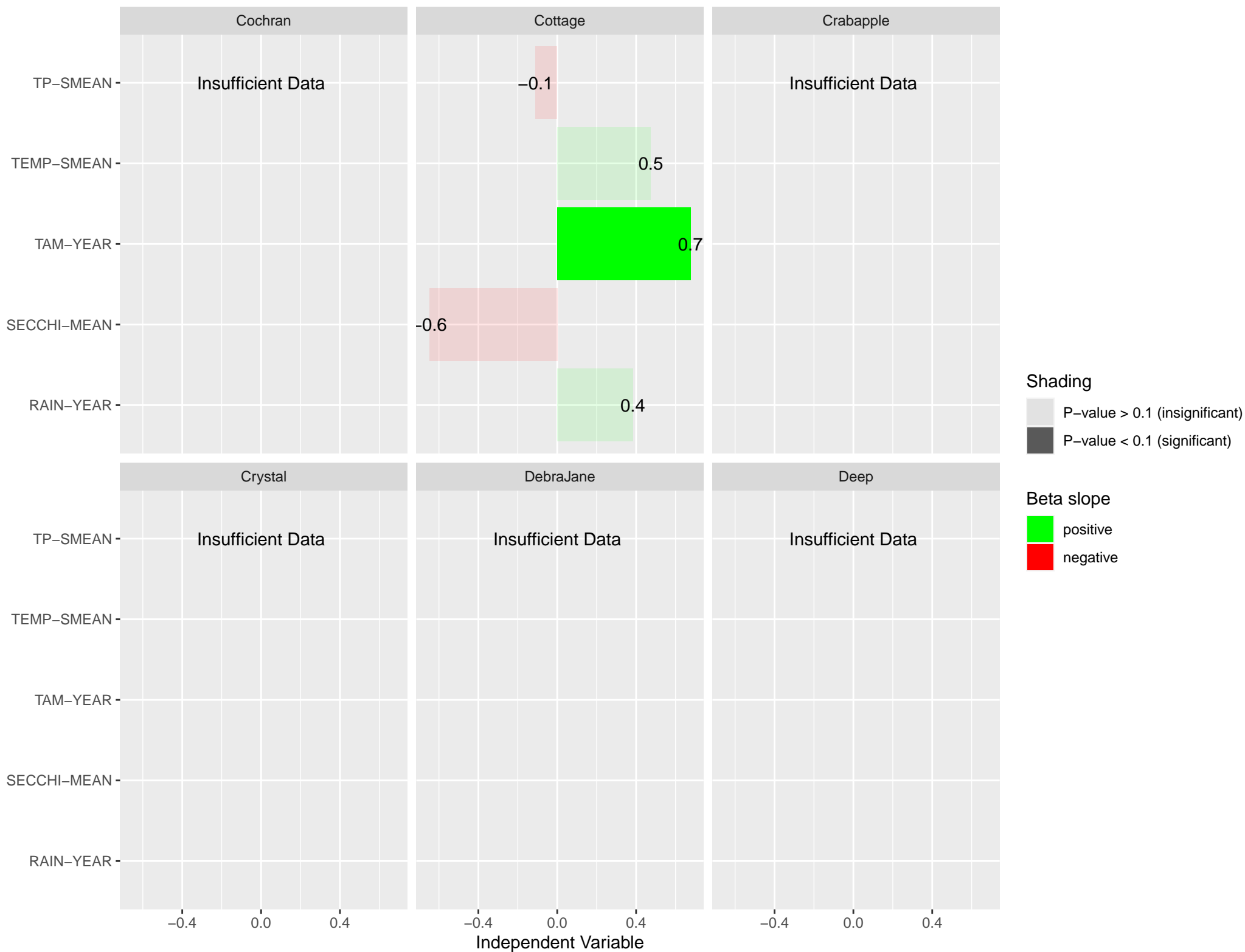
Multiple Regression Results for CHLA-SMAX (Spring data)



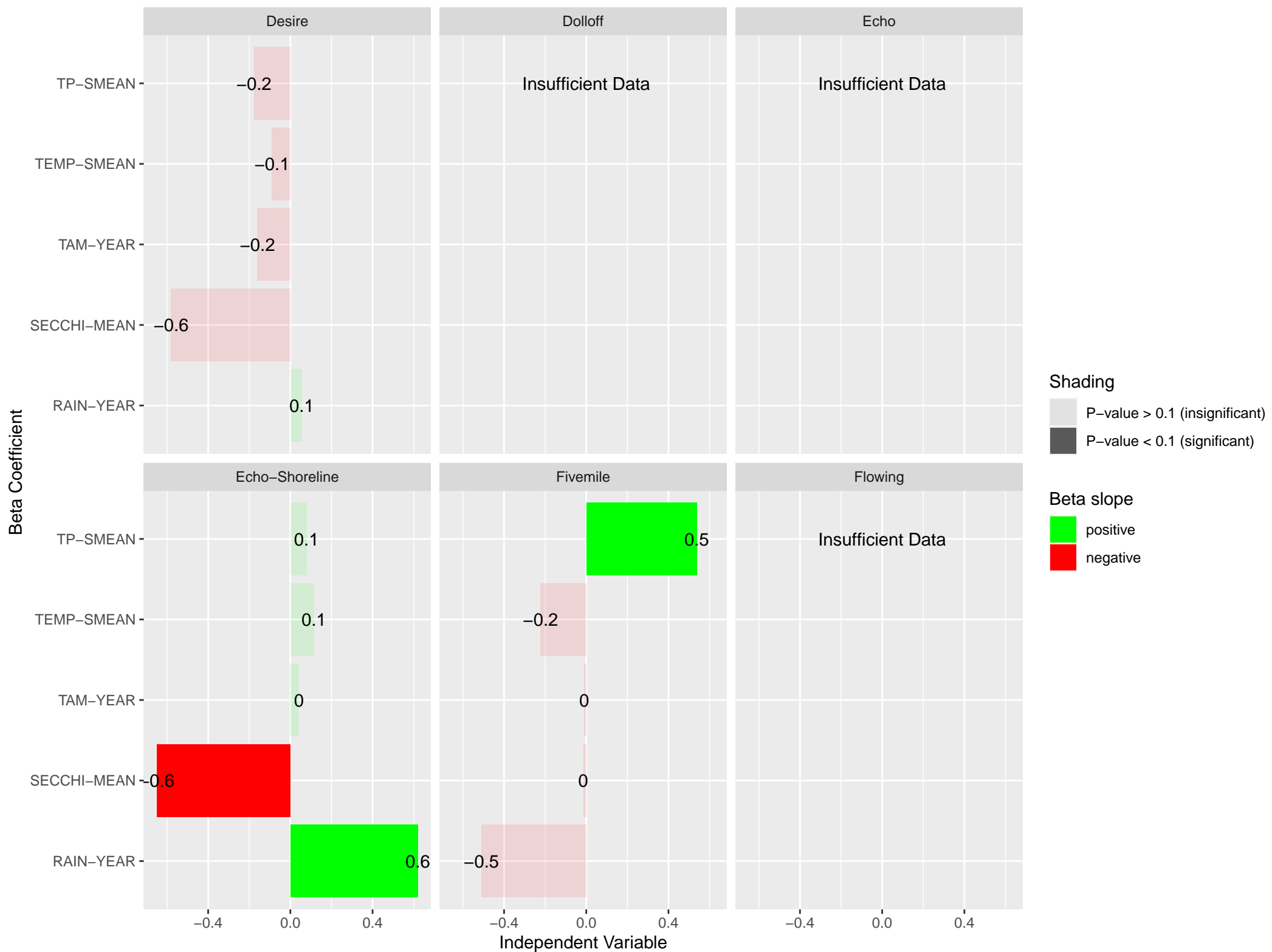
Multiple Regression Results for CHLA-SMAX (Spring data)



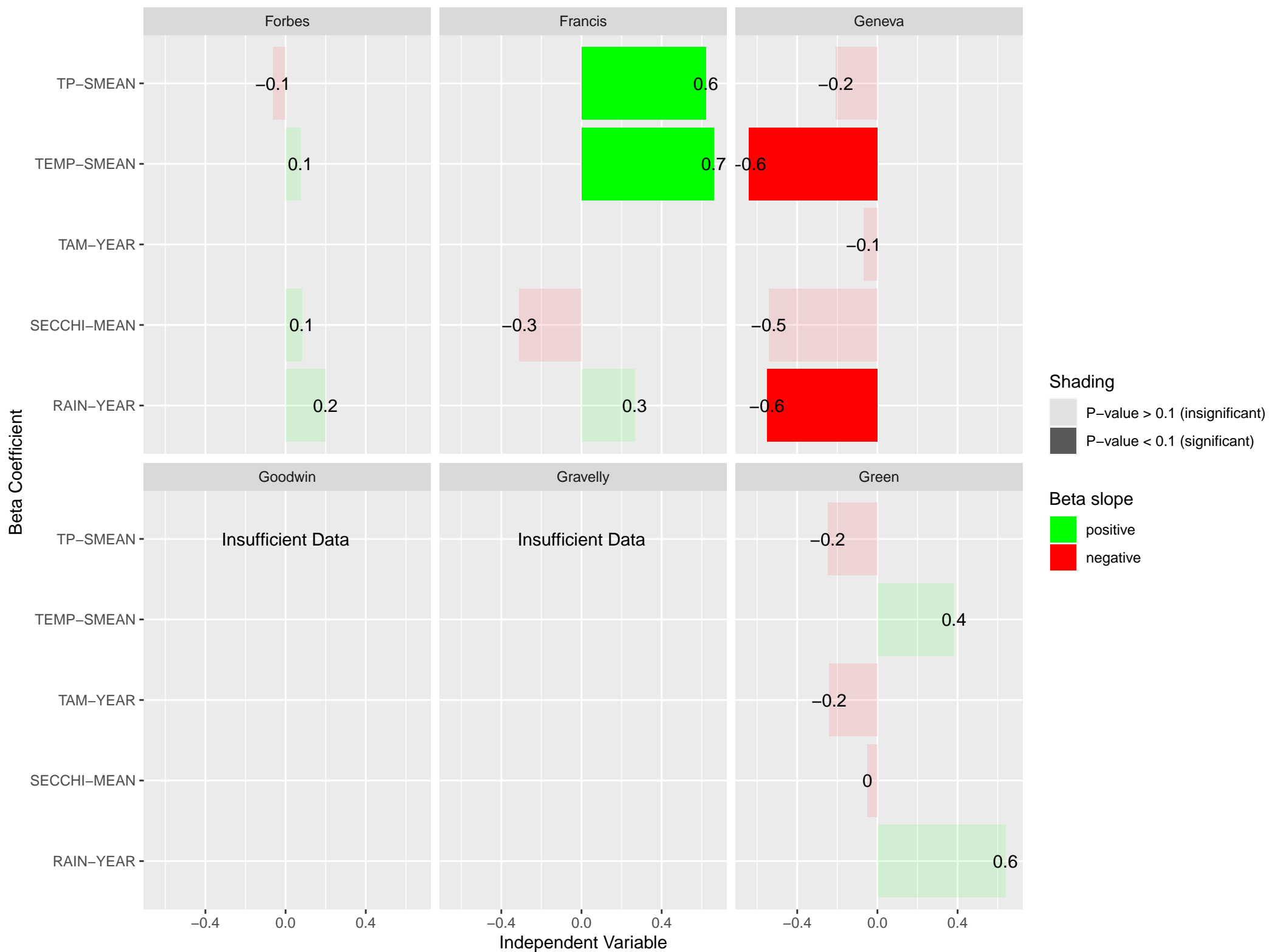
Multiple Regression Results for CHLA-SMAX (Spring data)



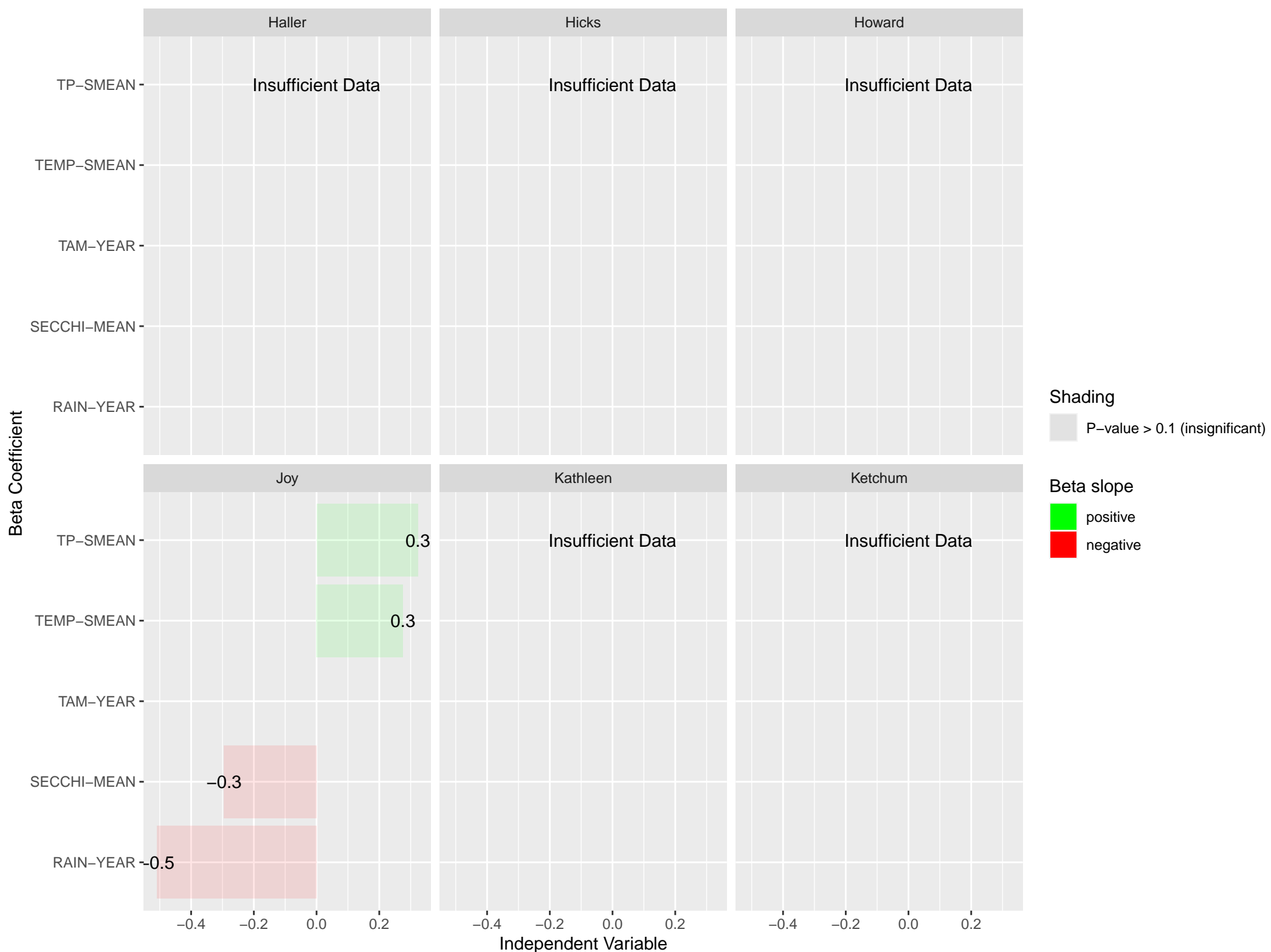
Multiple Regression Results for CHLA-SMAX (Spring data)



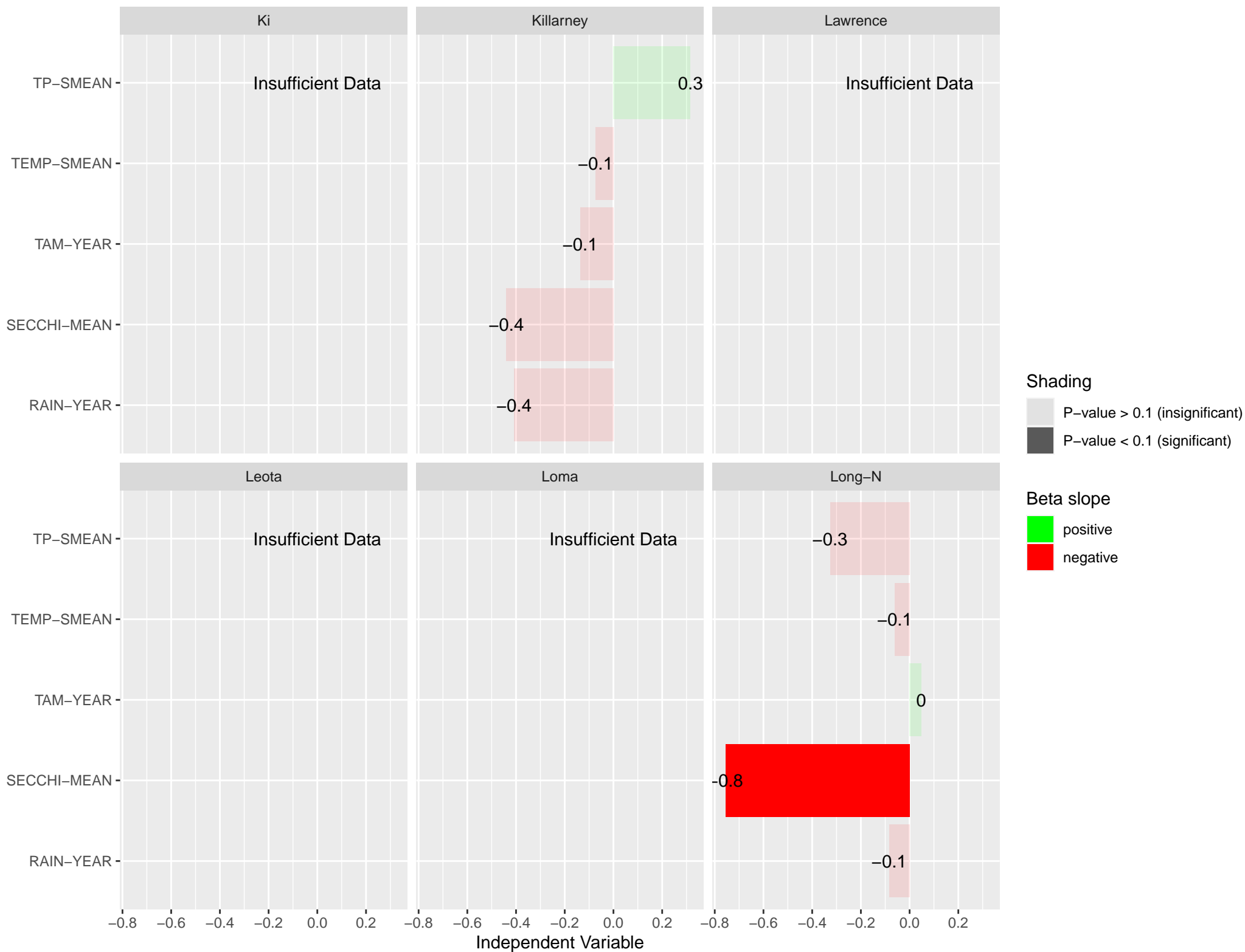
Multiple Regression Results for CHLA-SMAX (Spring data)



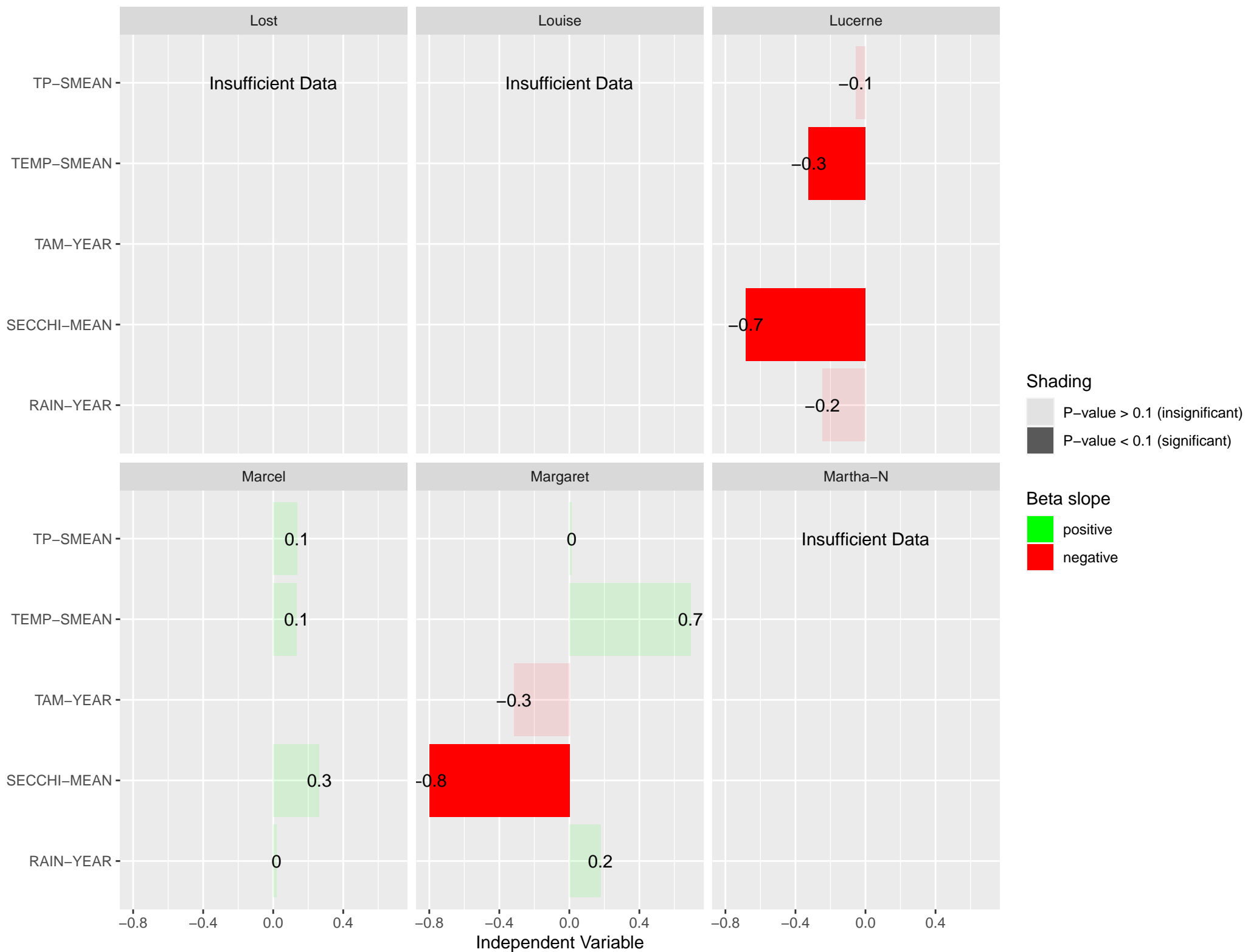
Multiple Regression Results for CHLA-SMAX (Spring data)



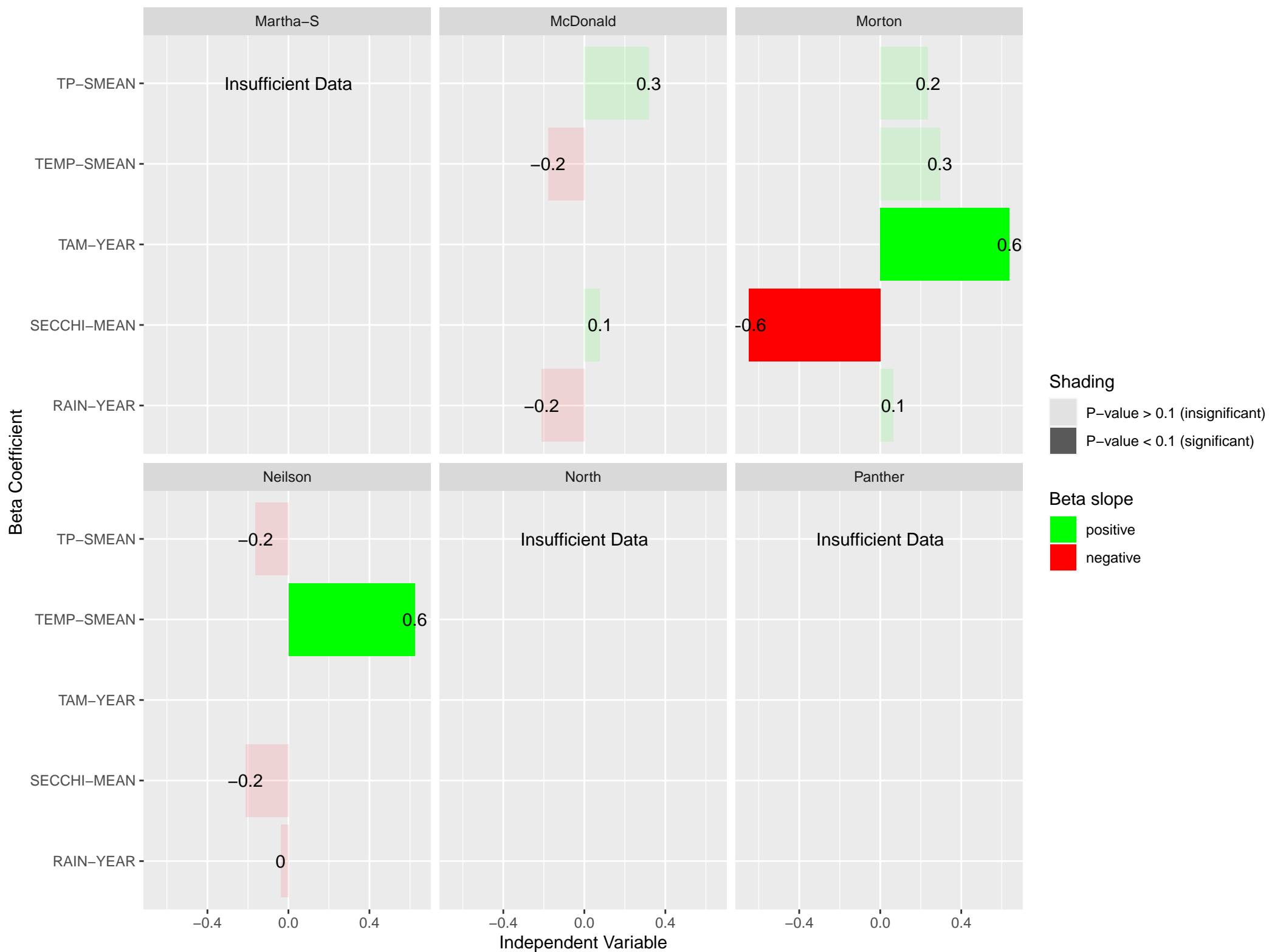
Multiple Regression Results for CHLA-SMAX (Spring data)



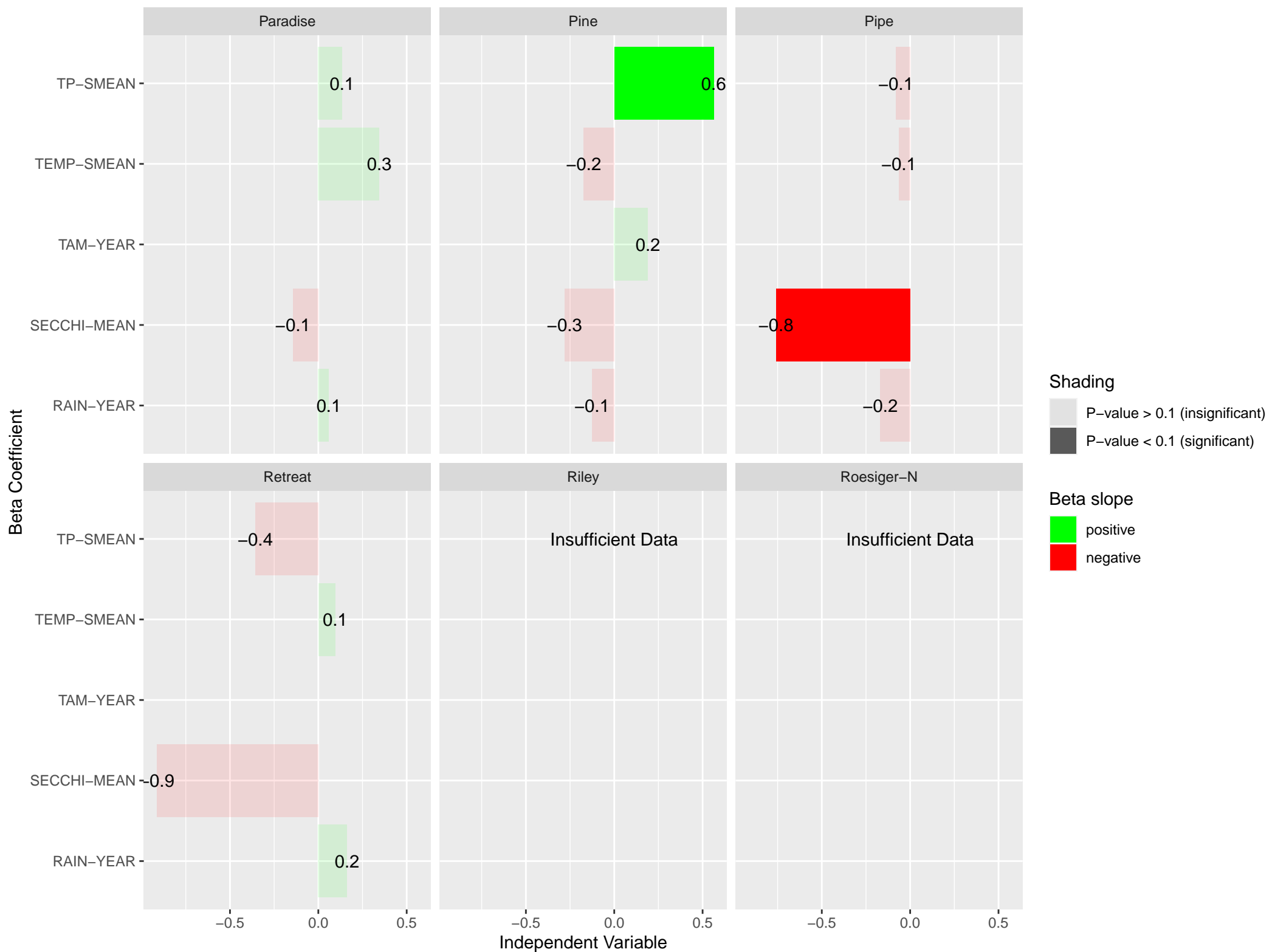
Multiple Regression Results for CHLA-SMAX (Spring data)



Multiple Regression Results for CHLA-SMAX (Spring data)



Multiple Regression Results for CHLA-SMAX (Spring data)



Multiple Regression Results for CHLA-SMAX (Spring data)

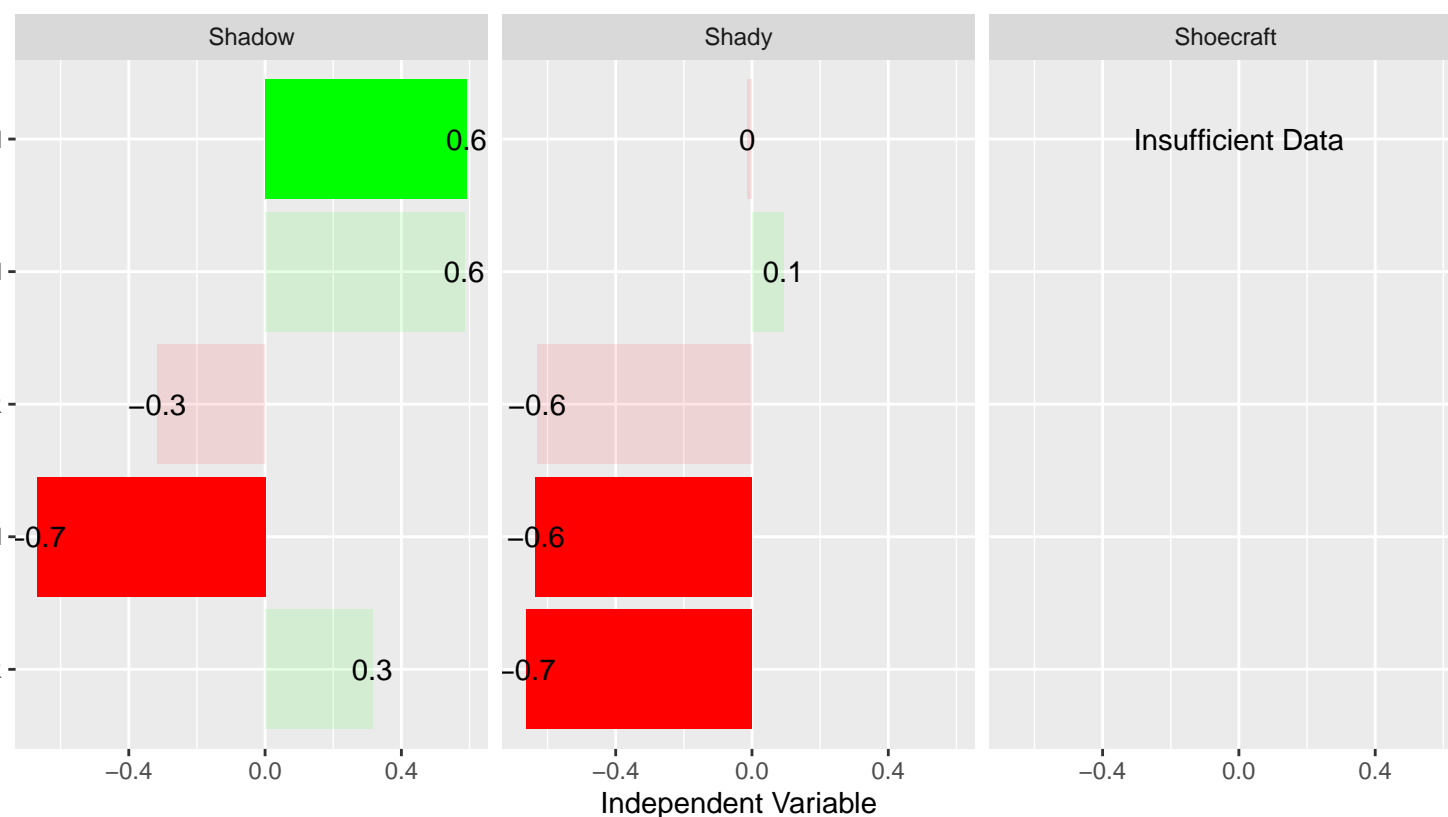


Shading

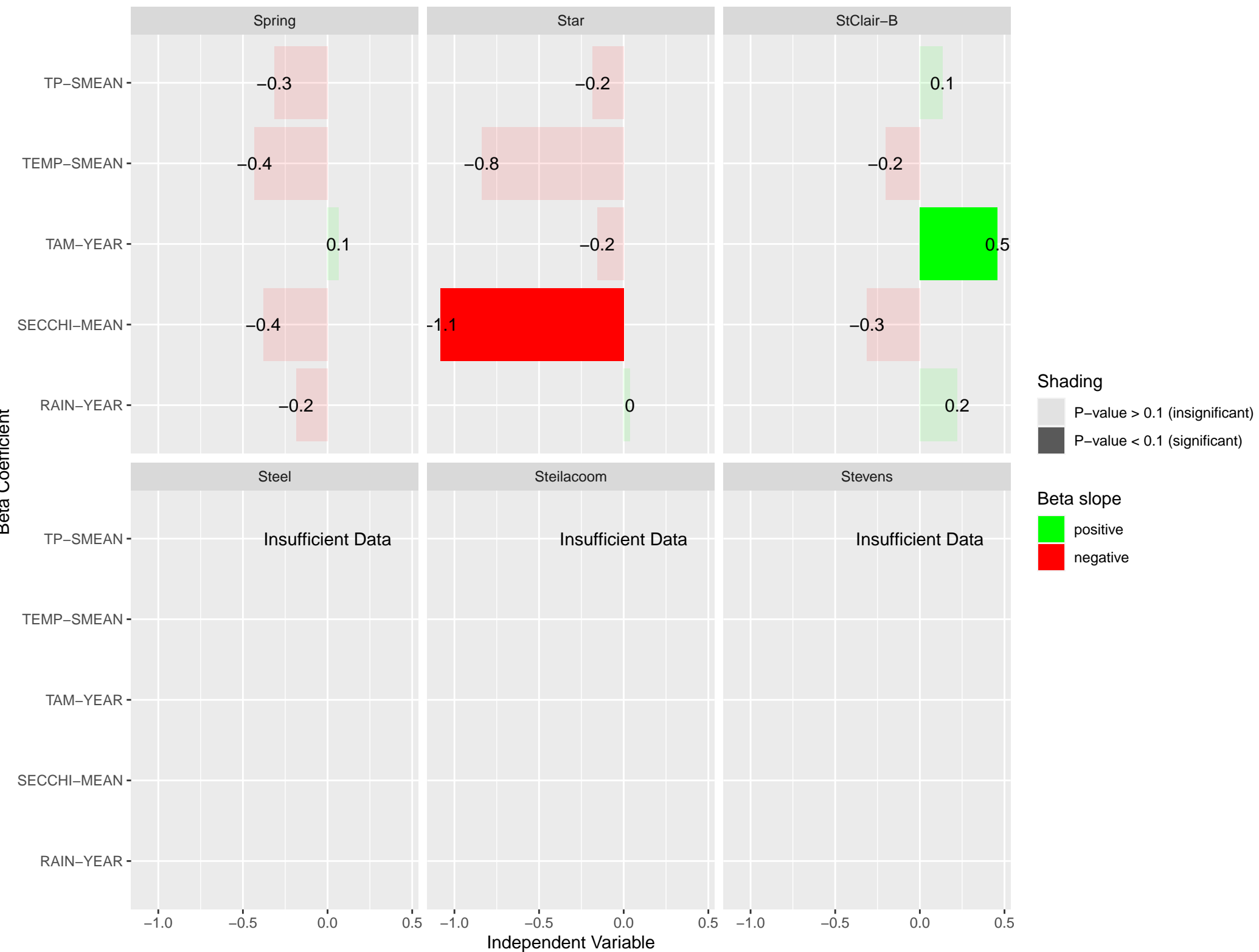
- P-value > 0.1 (insignificant)
- P-value < 0.1 (significant)

Beta slope

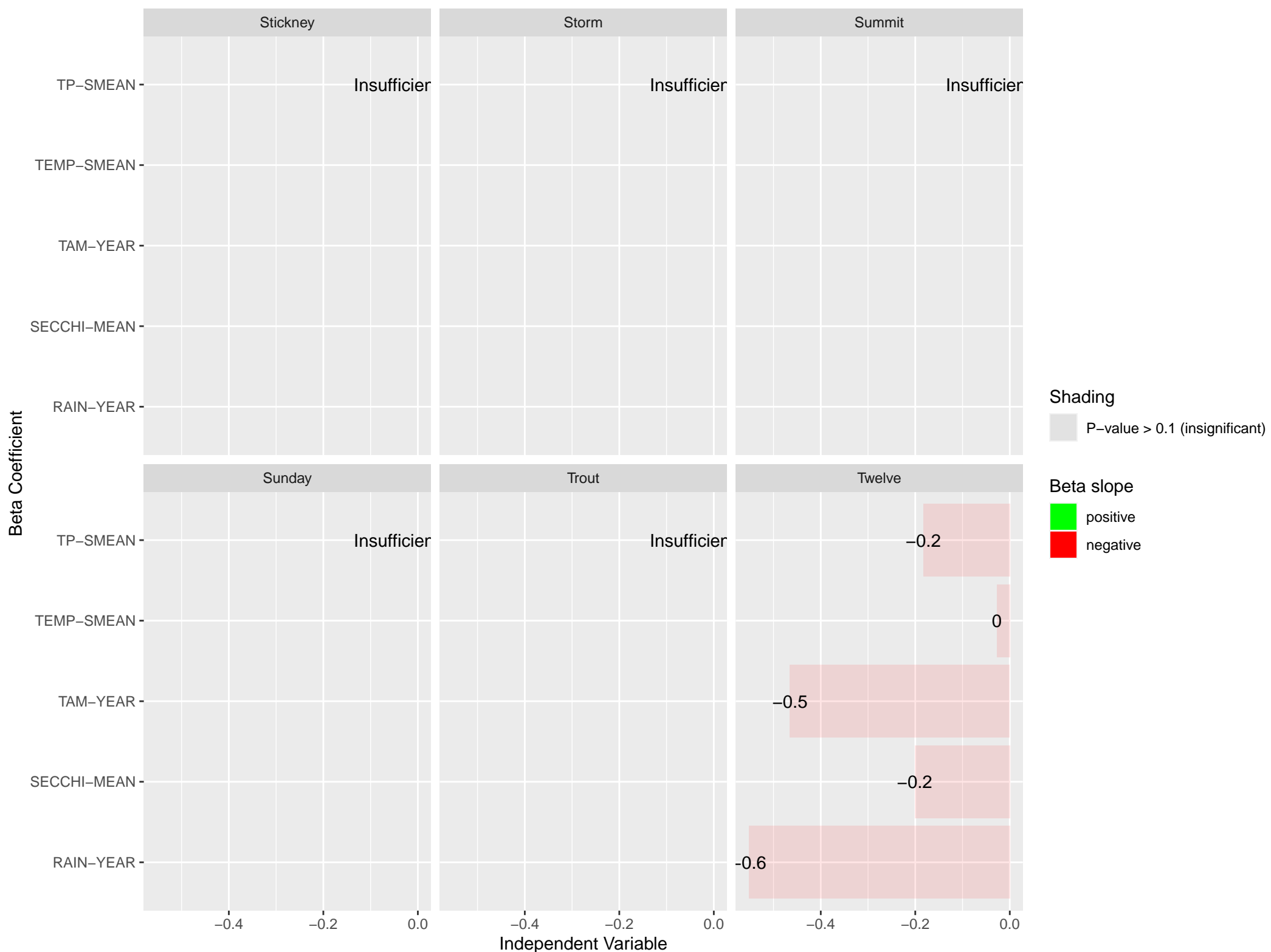
- positive
- negative



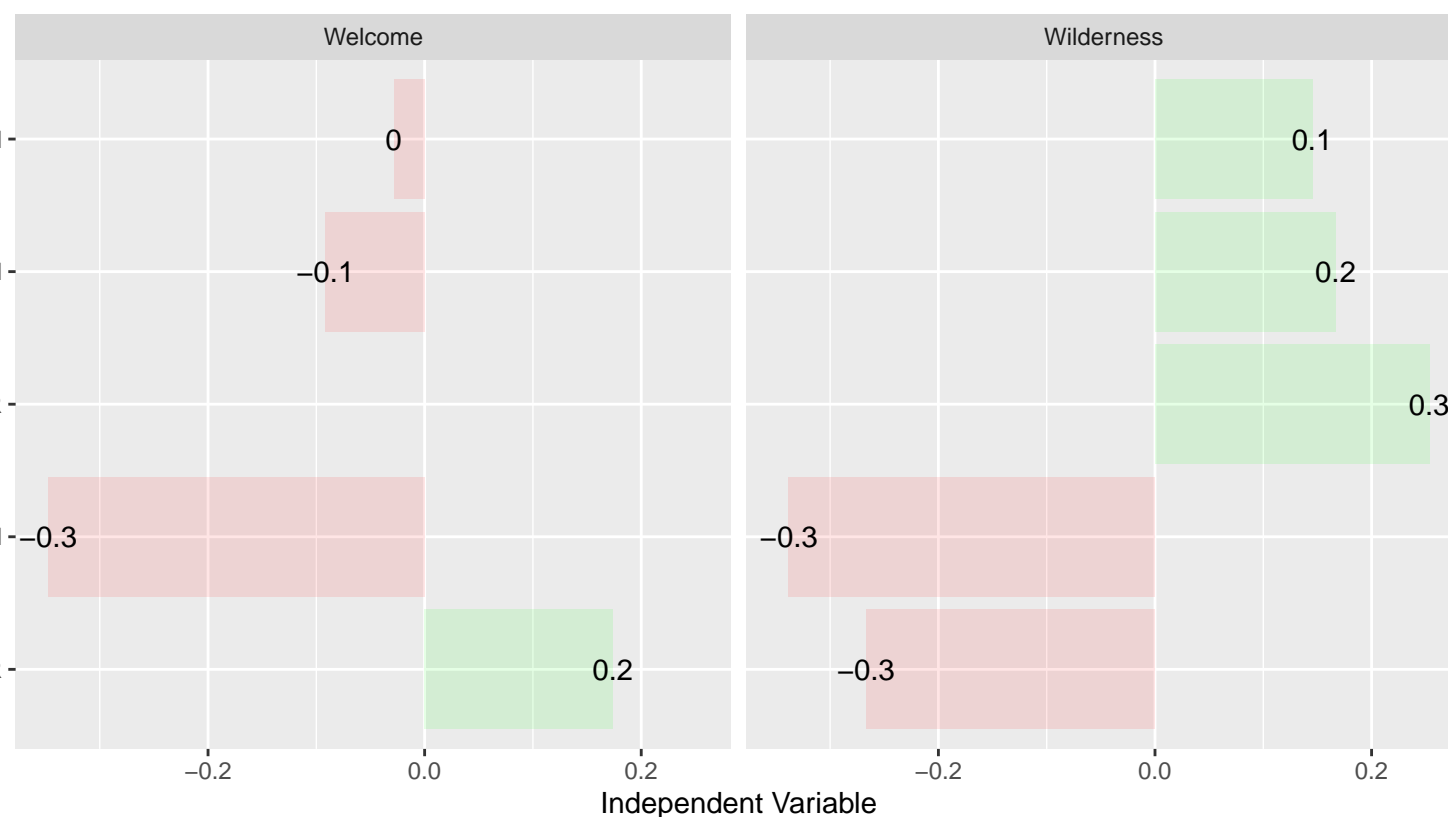
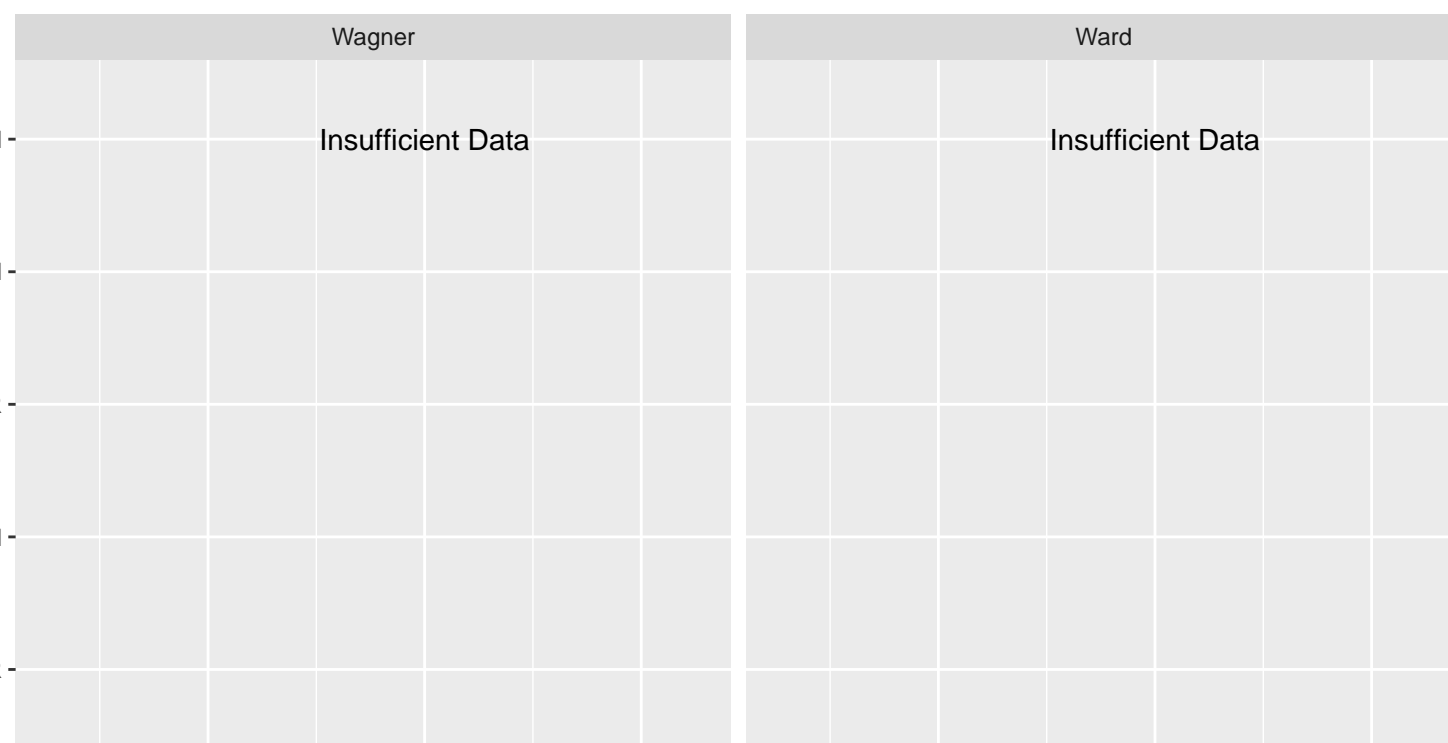
Multiple Regression Results for CHLA-SMAX (Spring data)



Multiple Regression Results for CHLA-SMAX (Spring data)



Multiple Regression Results for CHLA-SMAX (Spring data)



Shading

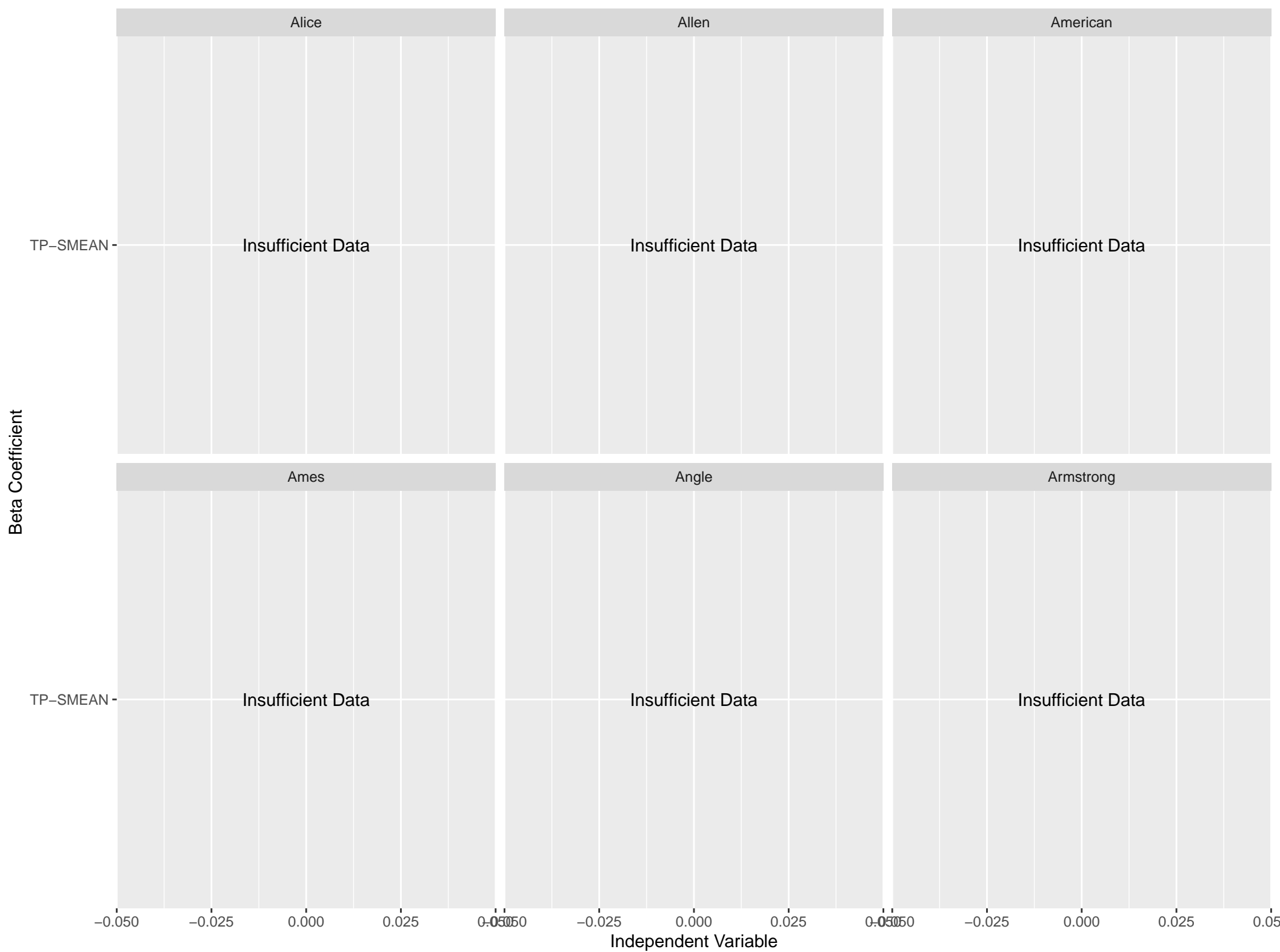
P-value > 0.1 (insignificant)

Beta slope

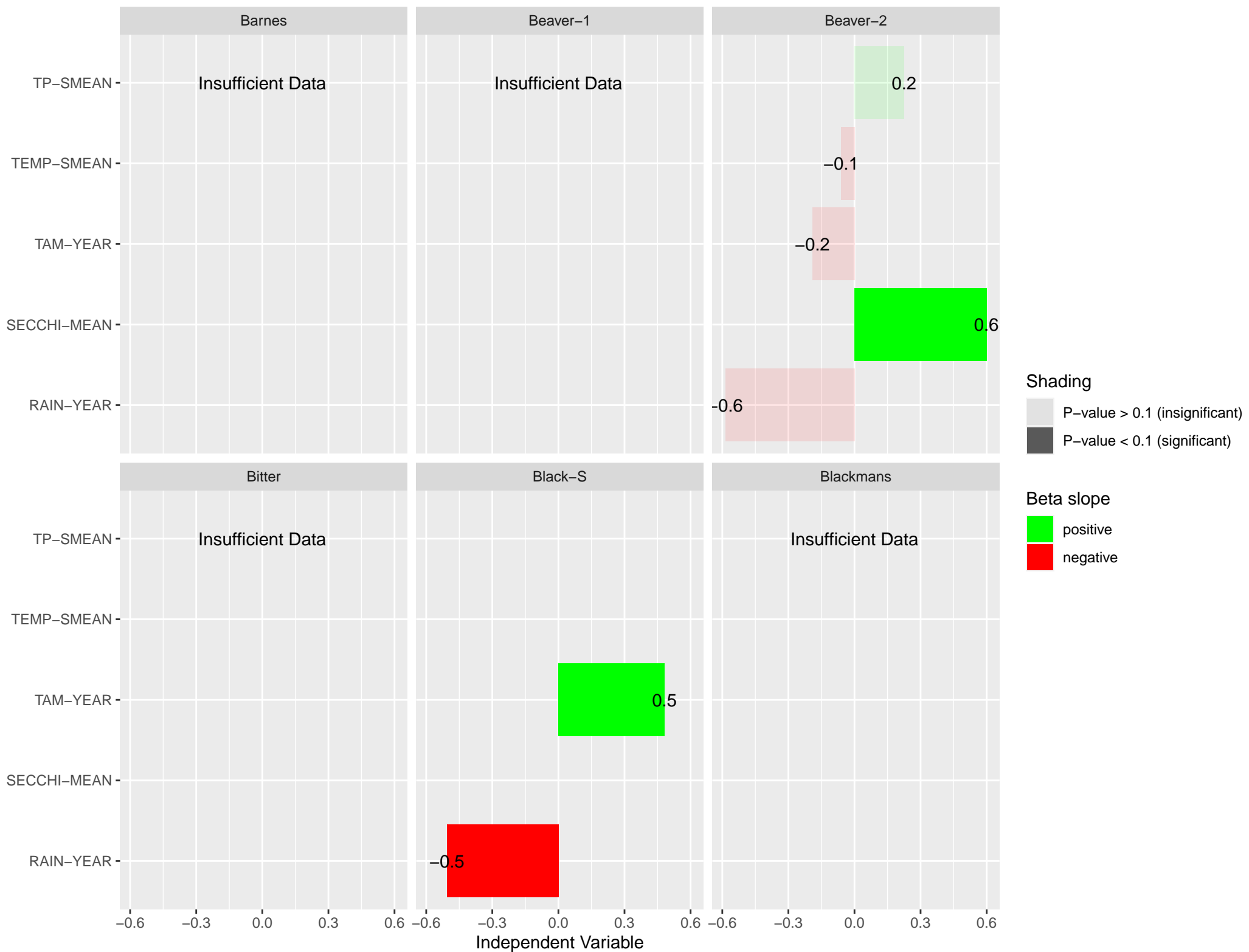
positive

negative

Multiple Regression Results for MC-MEAN (Spring data)



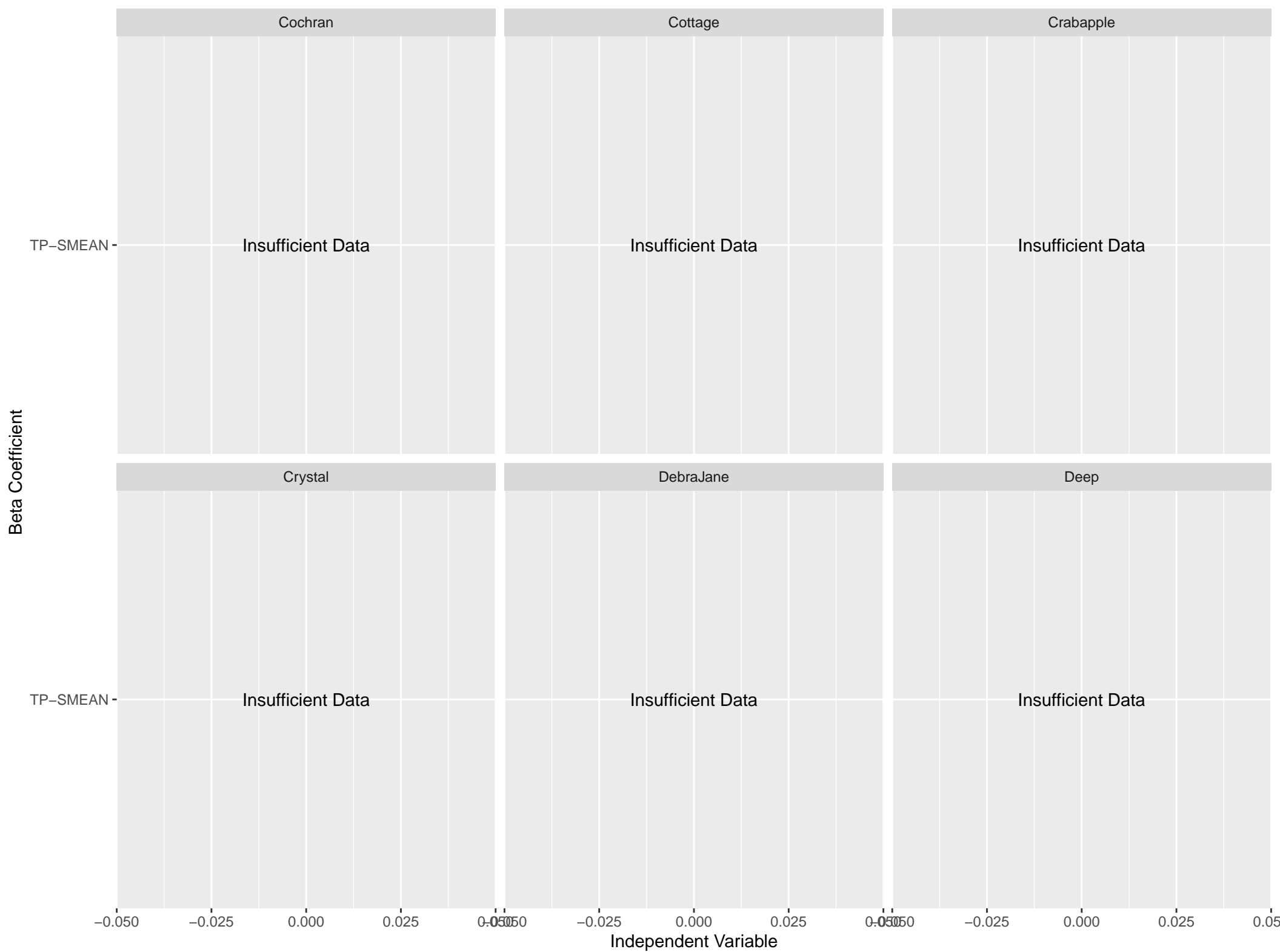
Multiple Regression Results for MC-MEAN (Spring data)



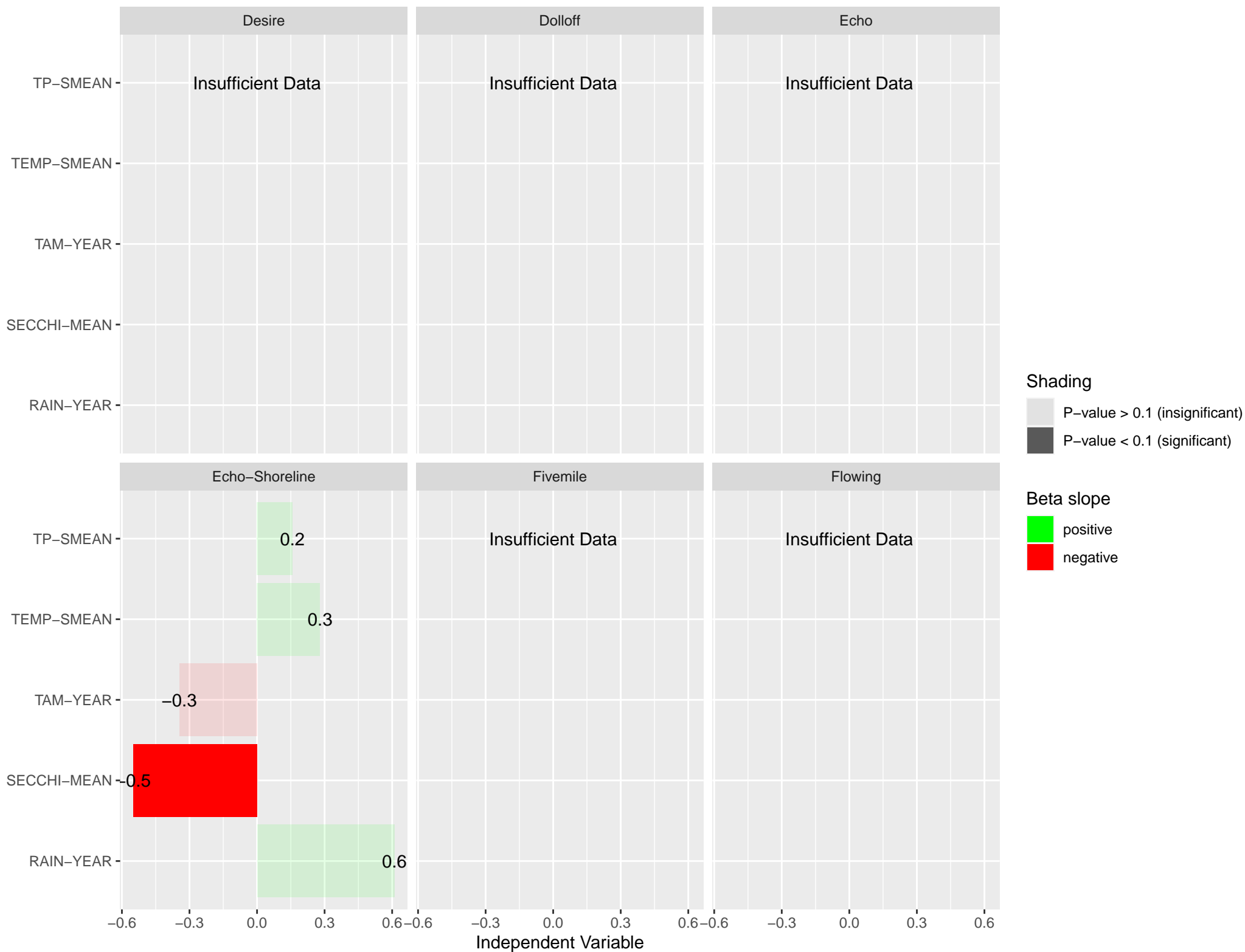
Multiple Regression Results for MC-MEAN (Spring data)



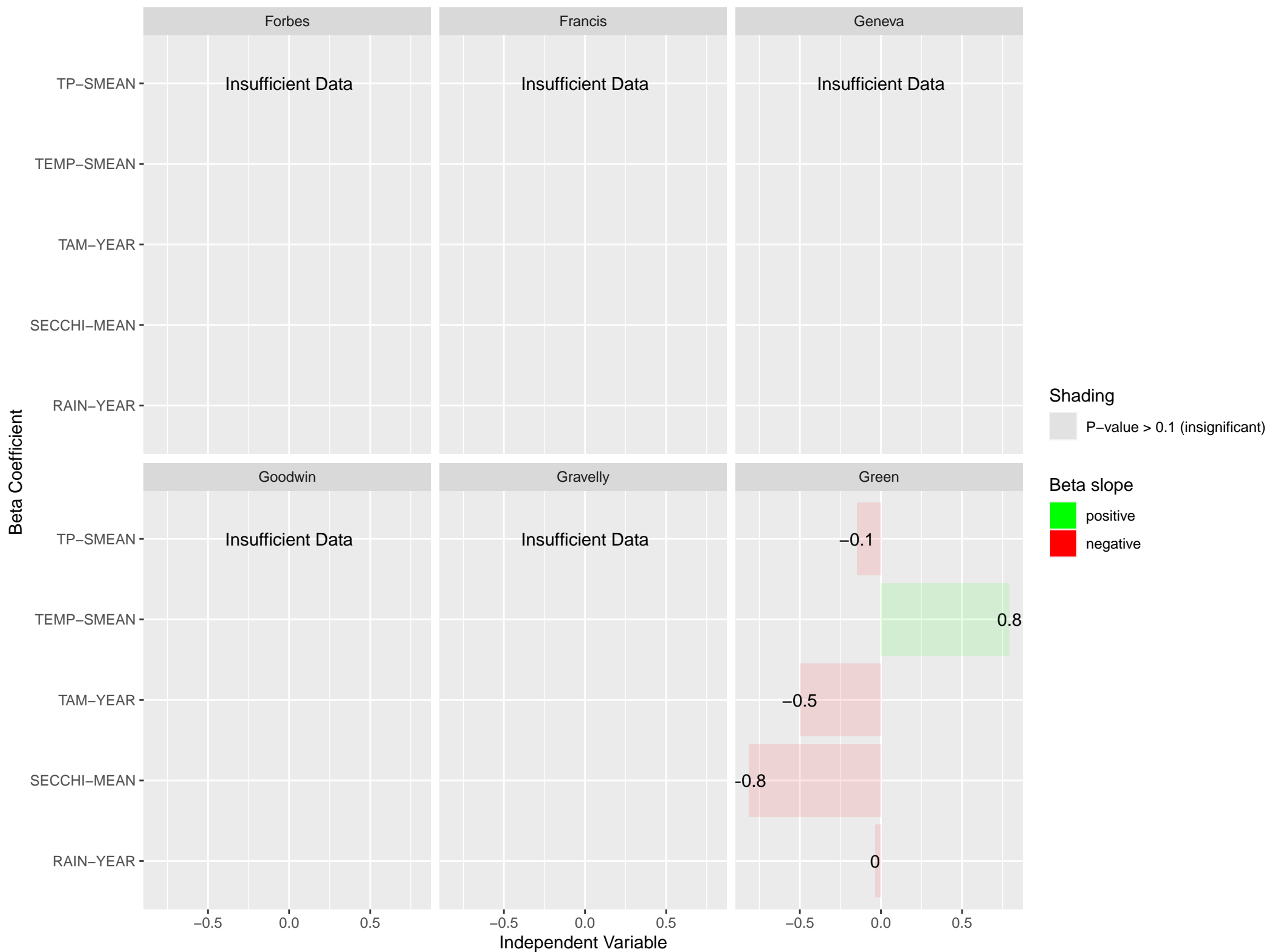
Multiple Regression Results for MC-MEAN (Spring data)



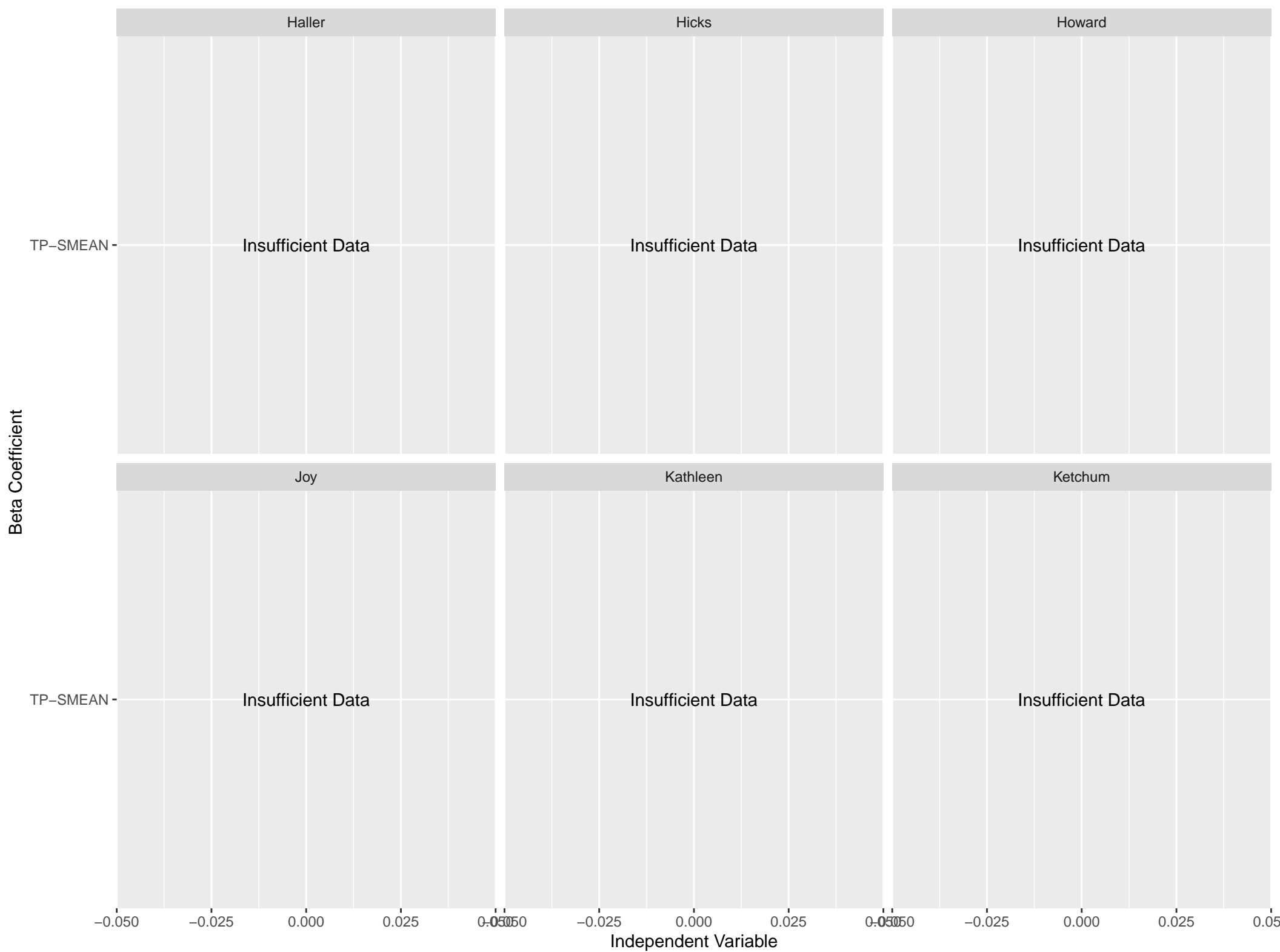
Multiple Regression Results for MC-MEAN (Spring data)



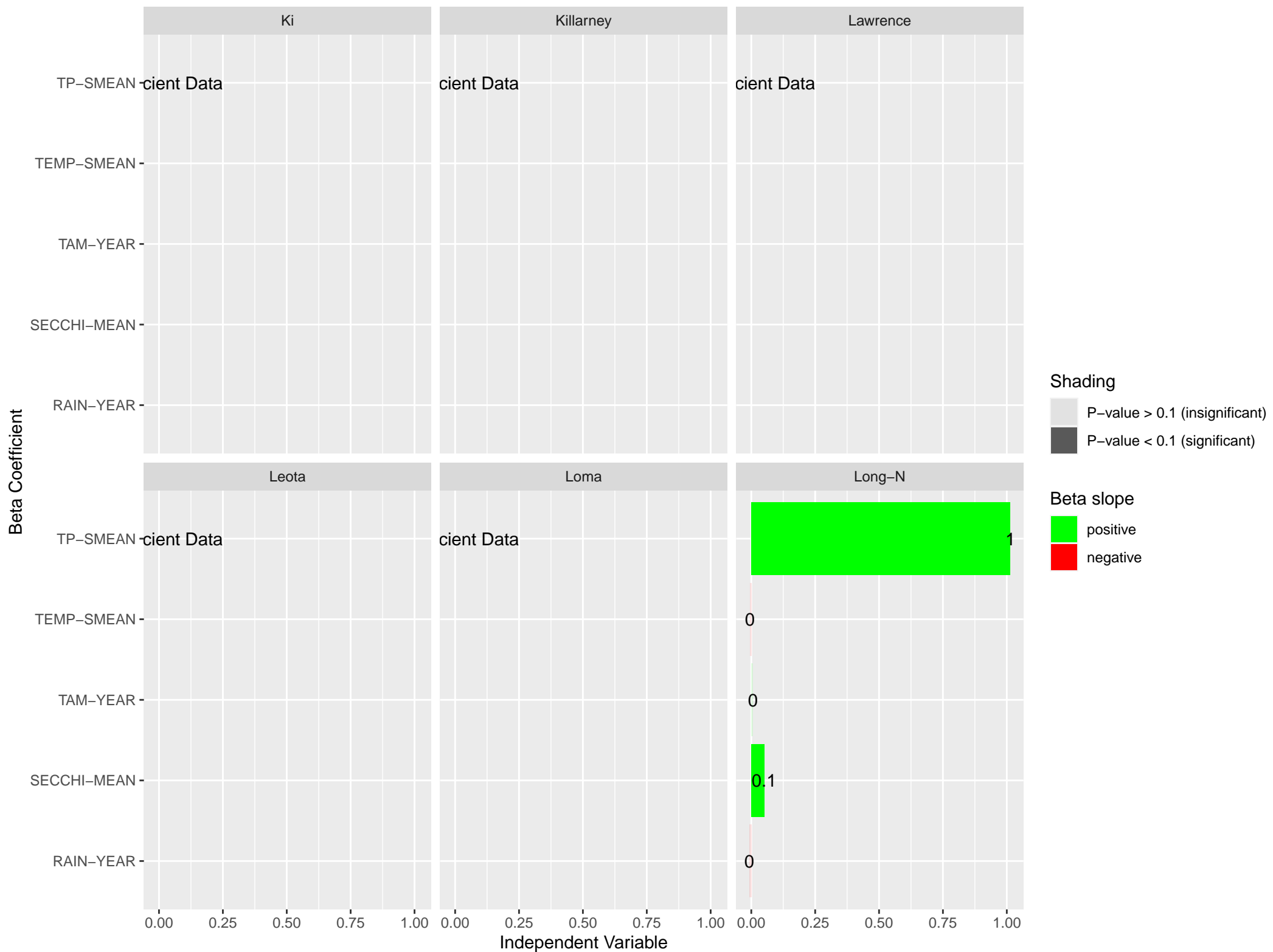
Multiple Regression Results for MC-MEAN (Spring data)



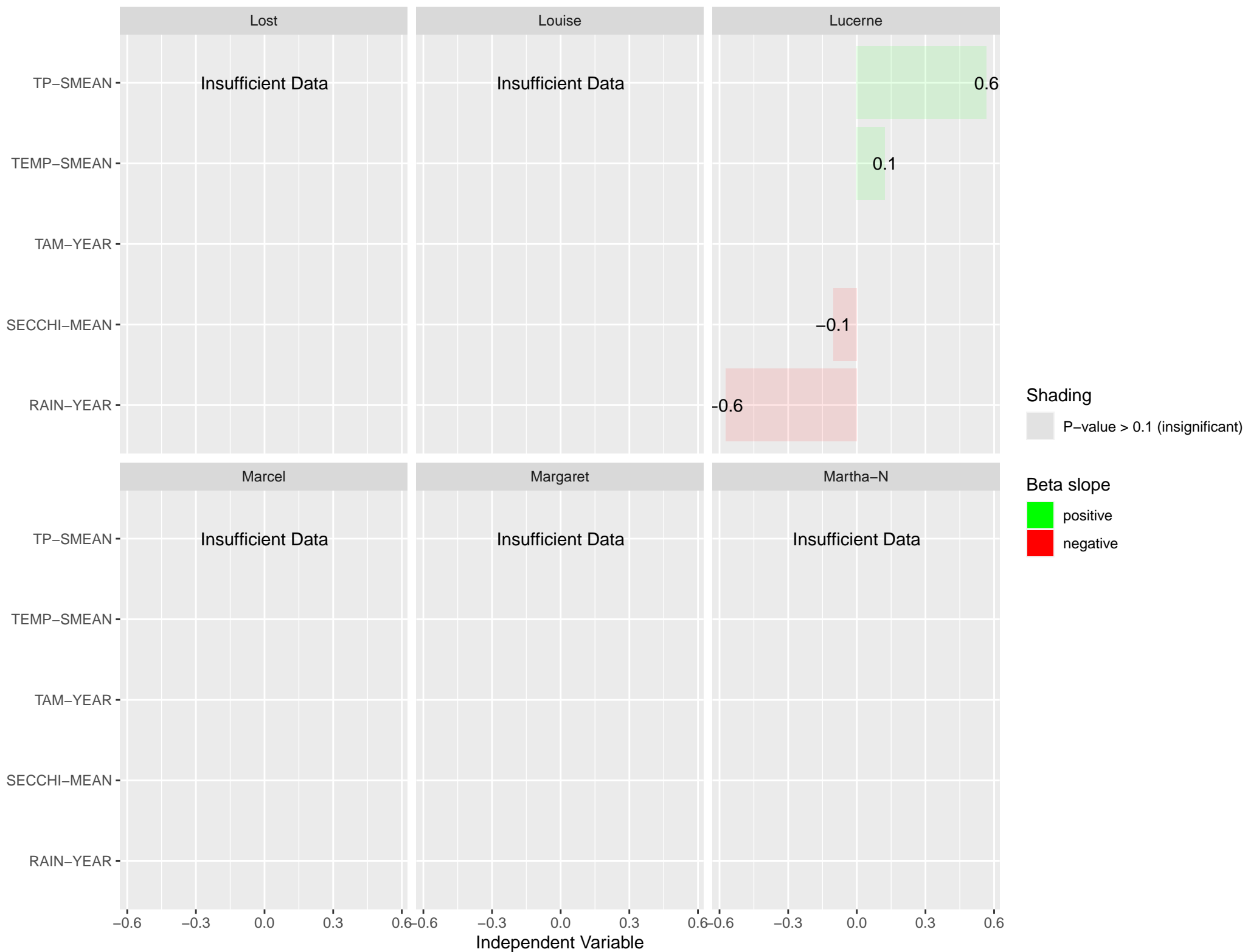
Multiple Regression Results for MC-MEAN (Spring data)



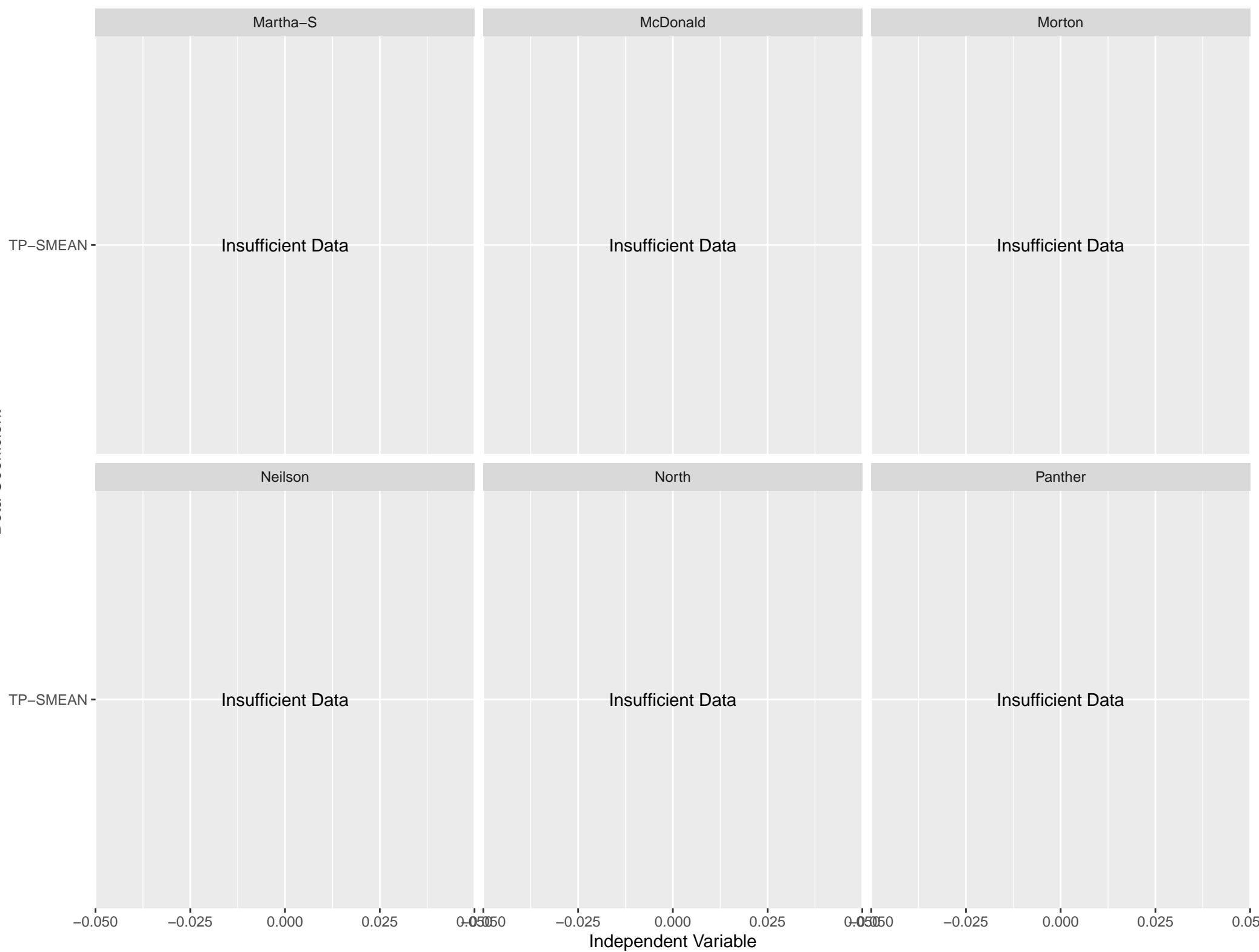
Multiple Regression Results for MC-MEAN (Spring data)



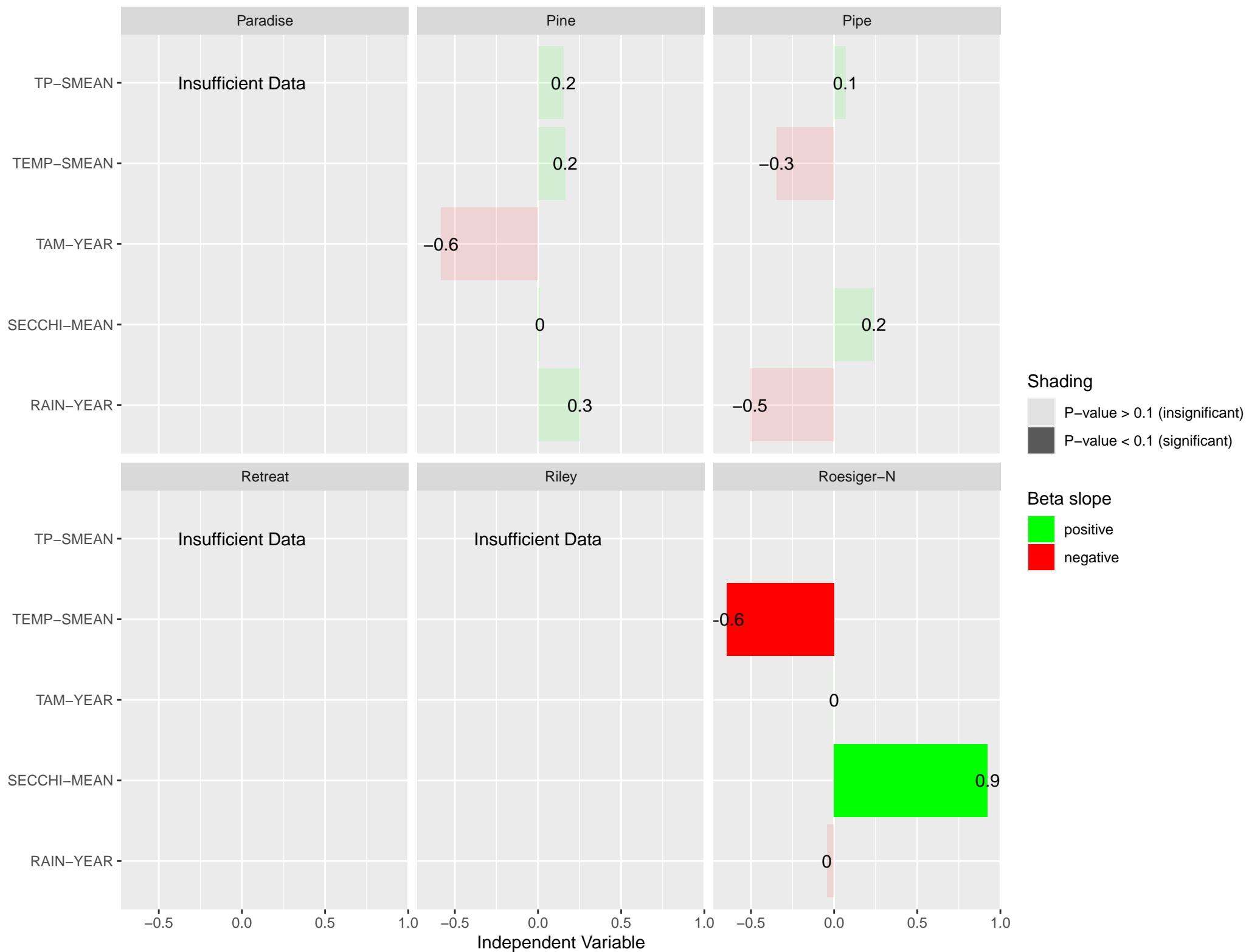
Multiple Regression Results for MC-MEAN (Spring data)



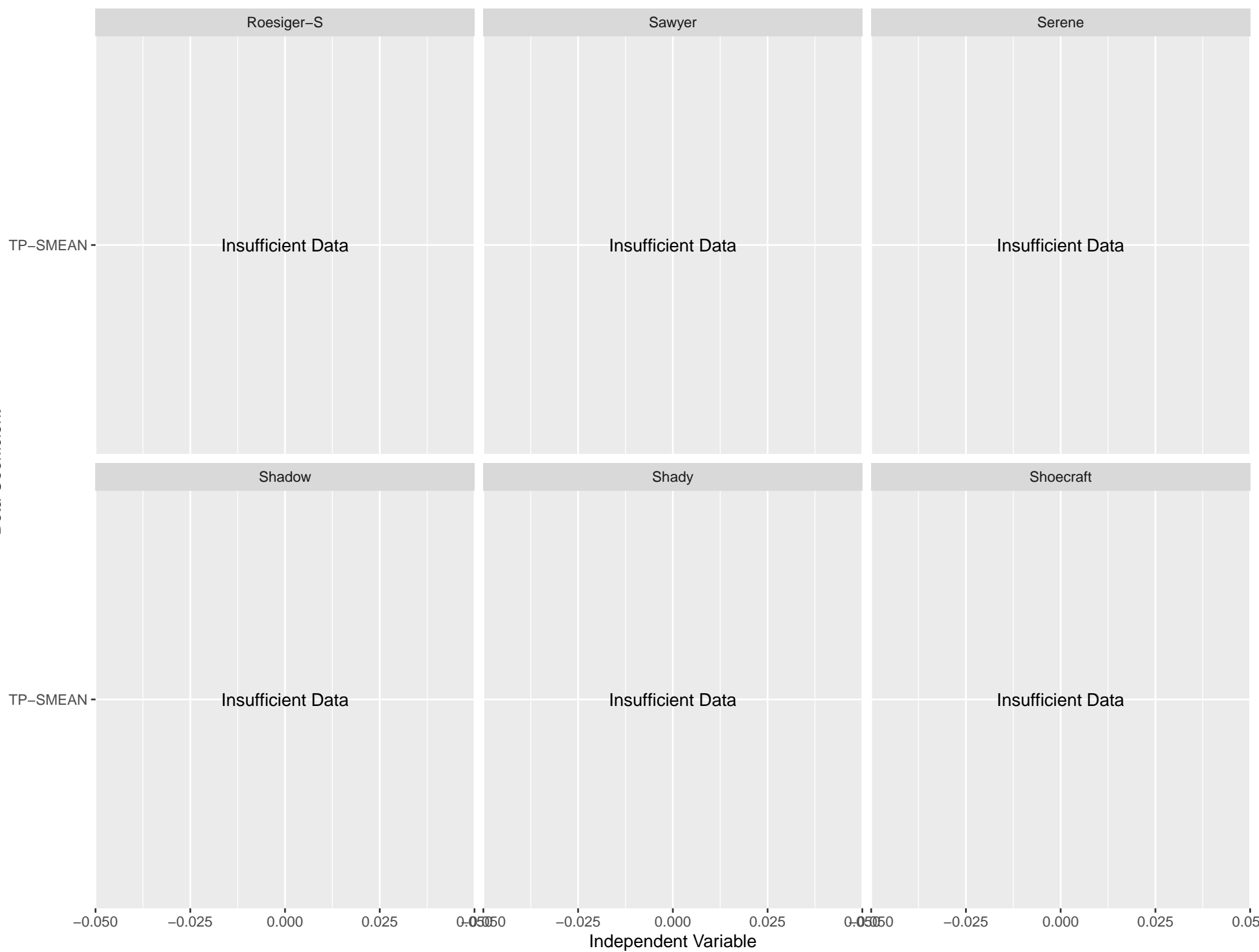
Multiple Regression Results for MC-MEAN (Spring data)



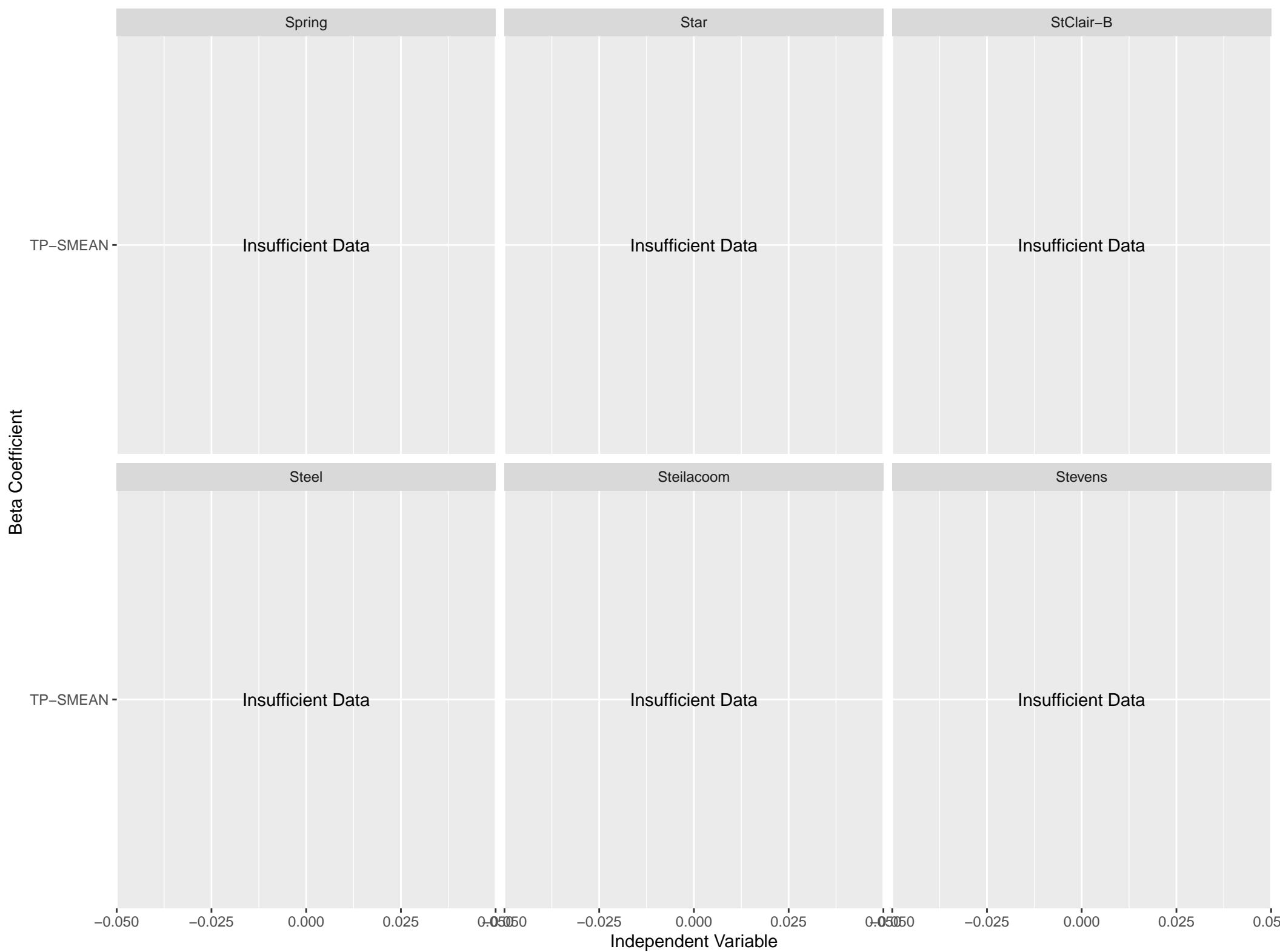
Multiple Regression Results for MC-MEAN (Spring data)



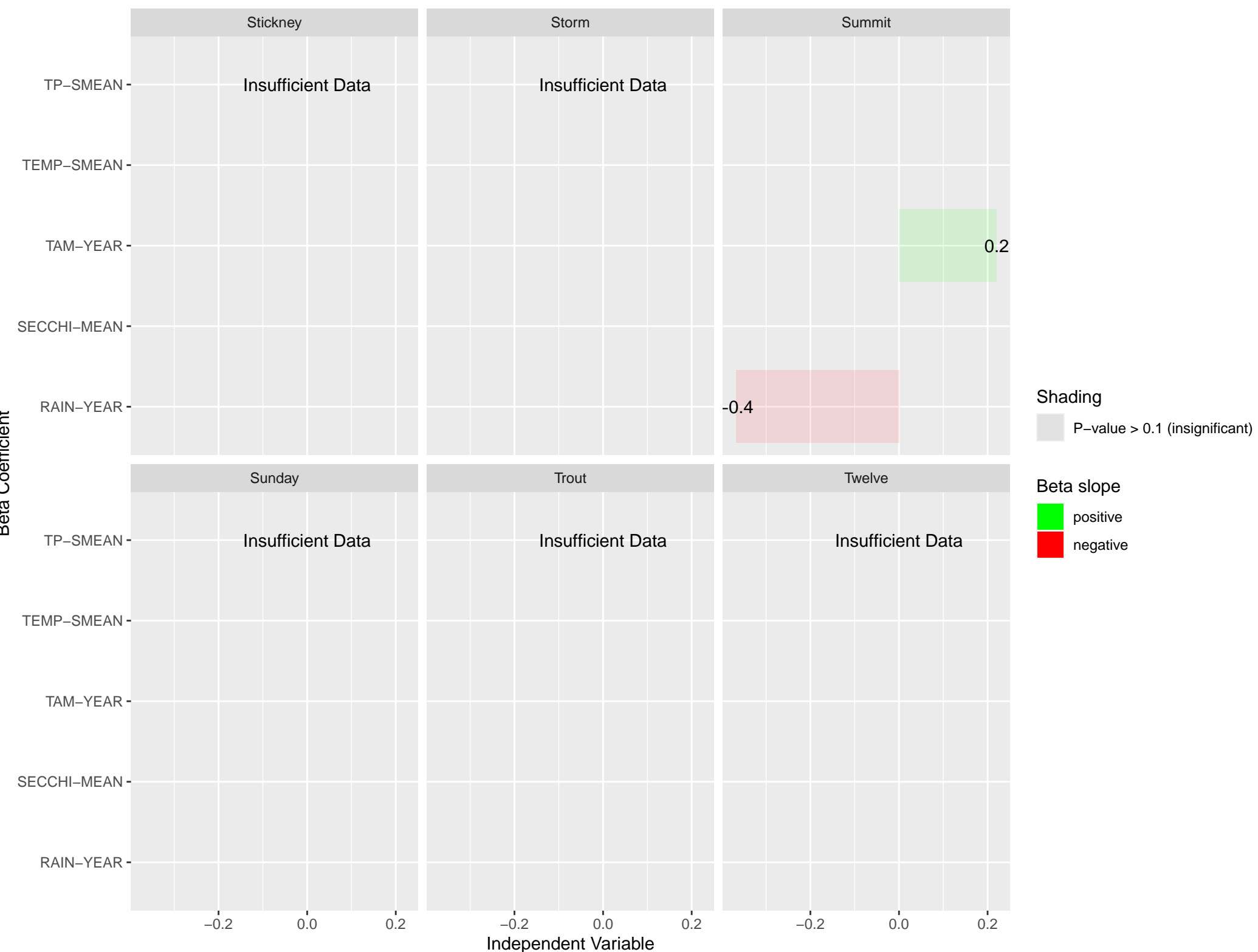
Multiple Regression Results for MC-MEAN (Spring data)



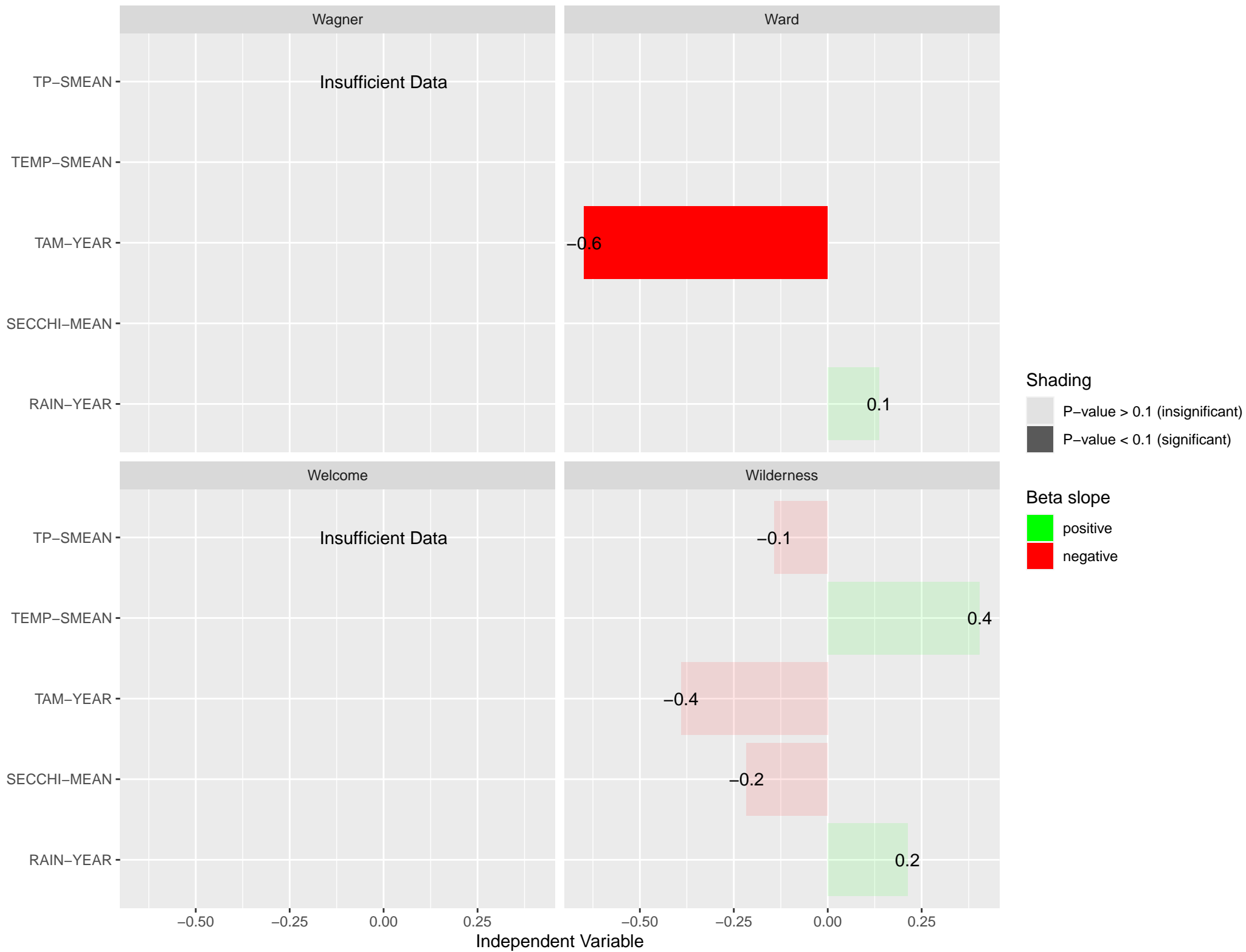
Multiple Regression Results for MC-MEAN (Spring data)



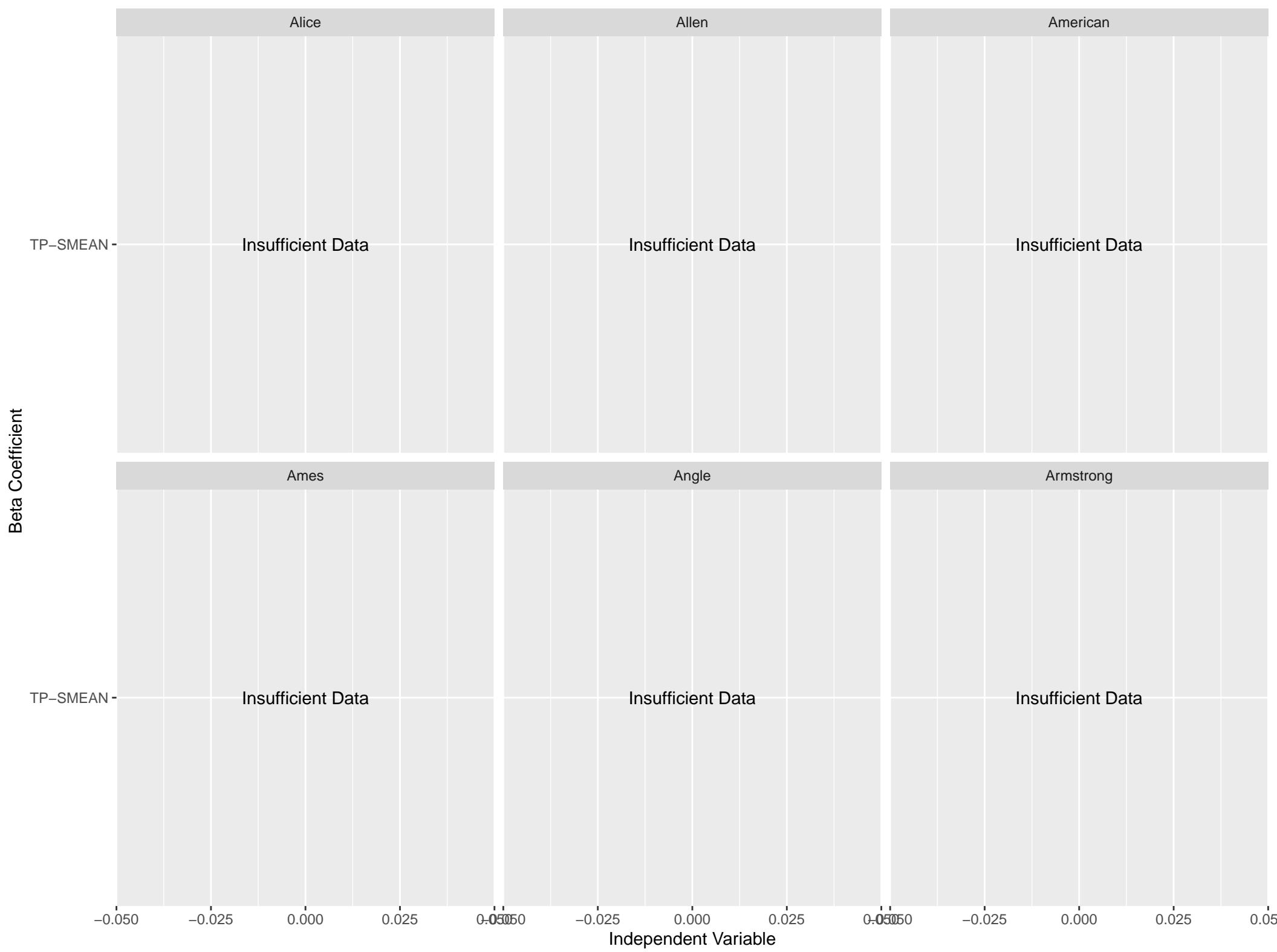
Multiple Regression Results for MC-MEAN (Spring data)



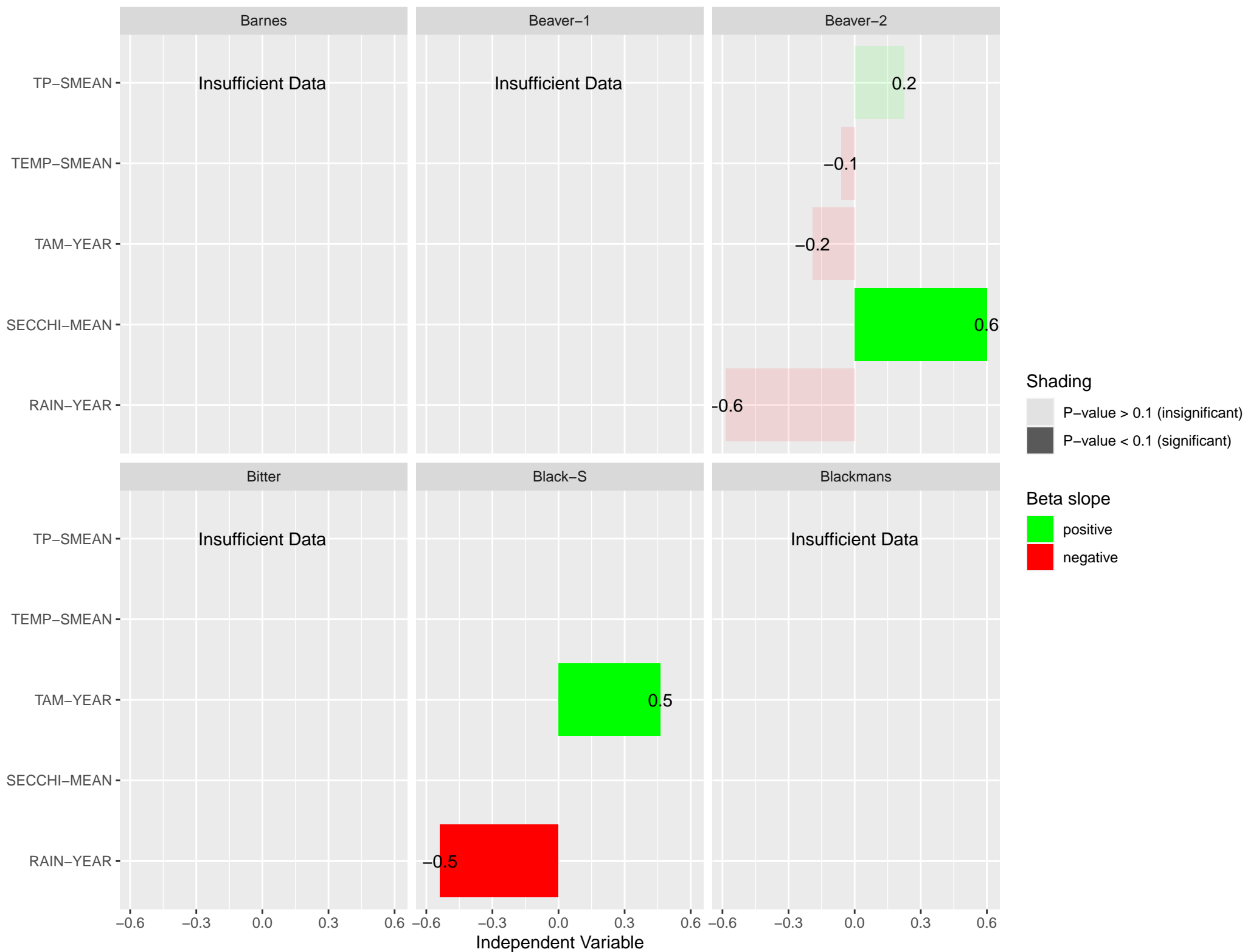
Multiple Regression Results for MC-MEAN (Spring data)



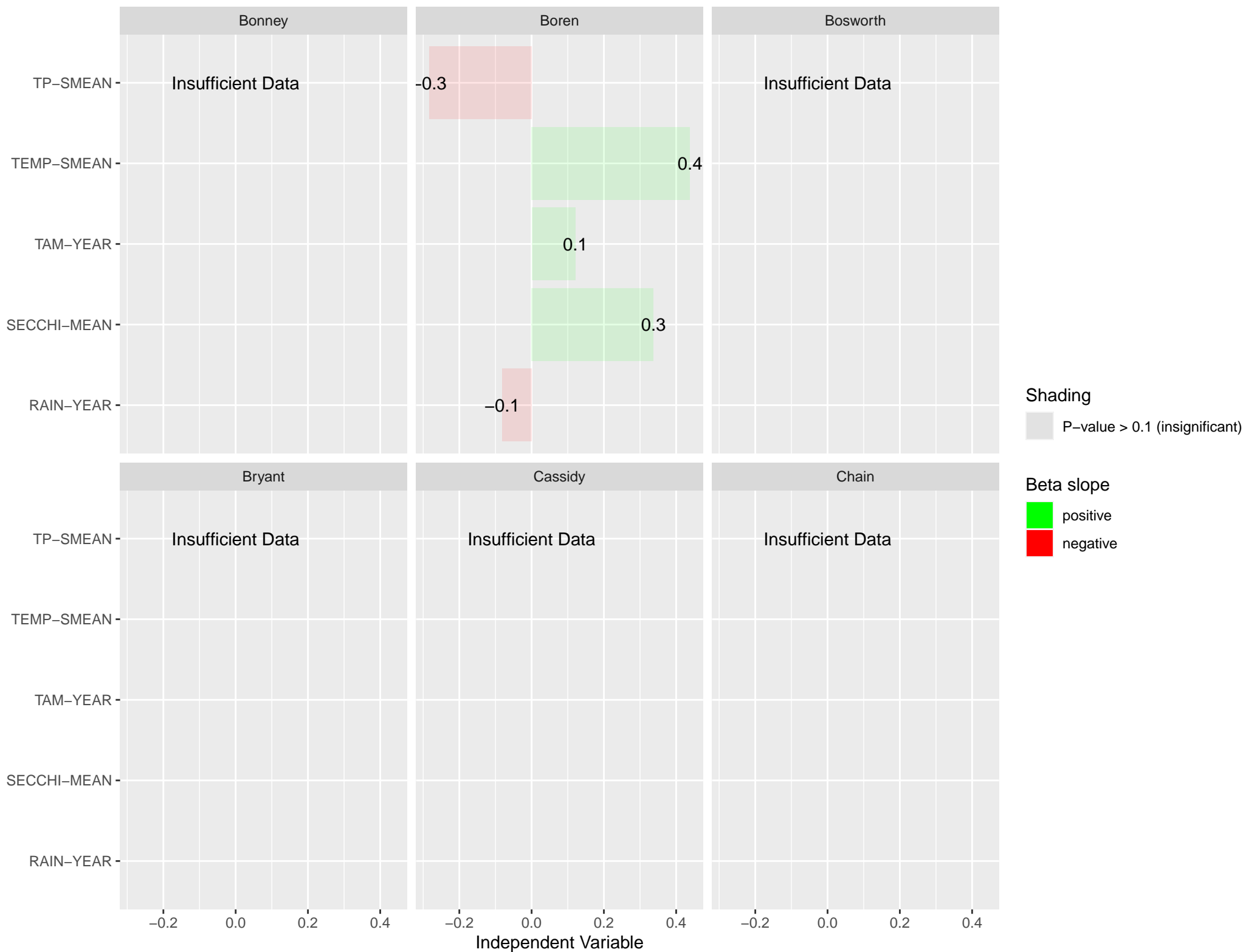
Multiple Regression Results for MC-MAX (Spring data)



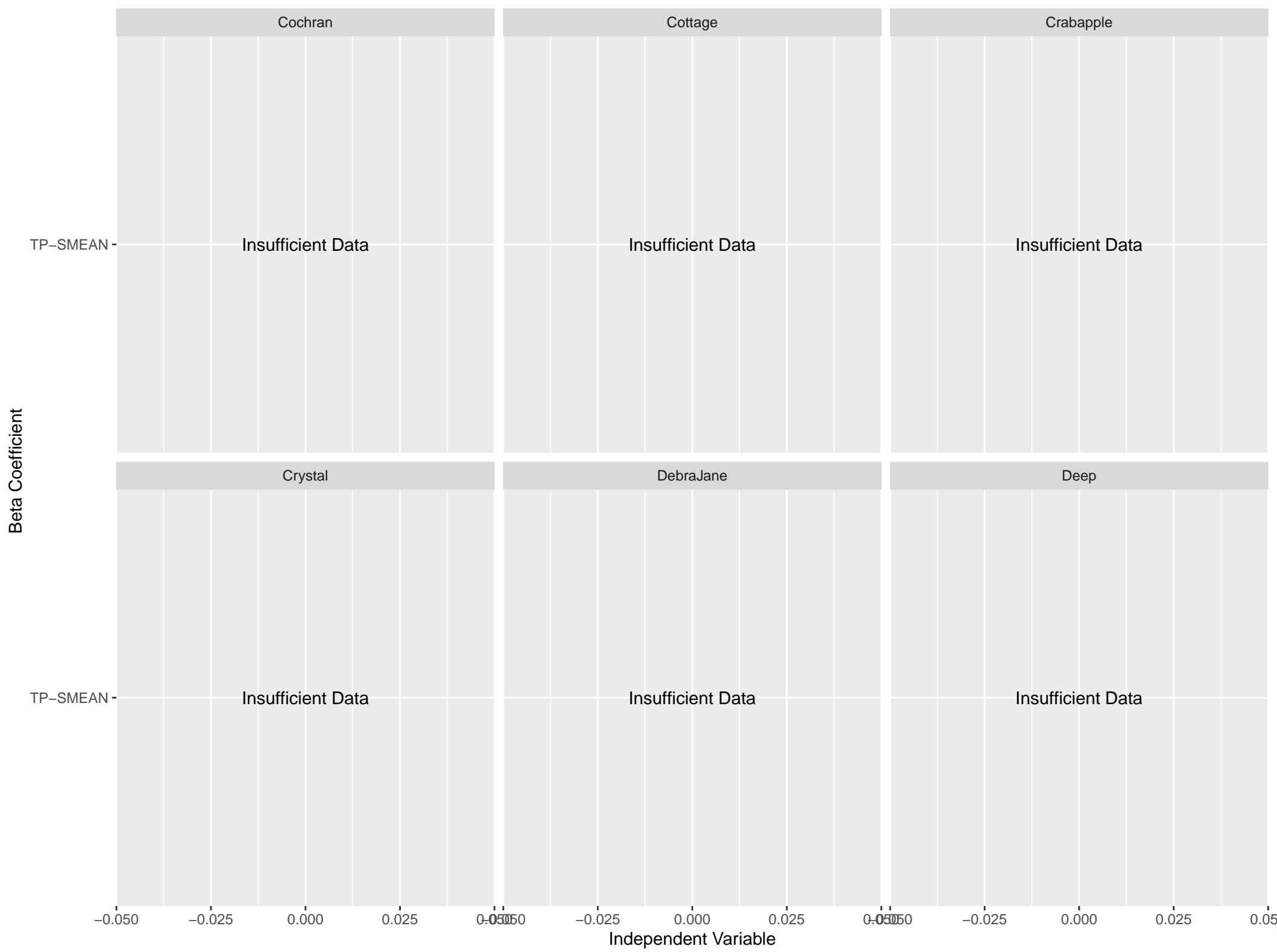
Multiple Regression Results for MC-MAX (Spring data)



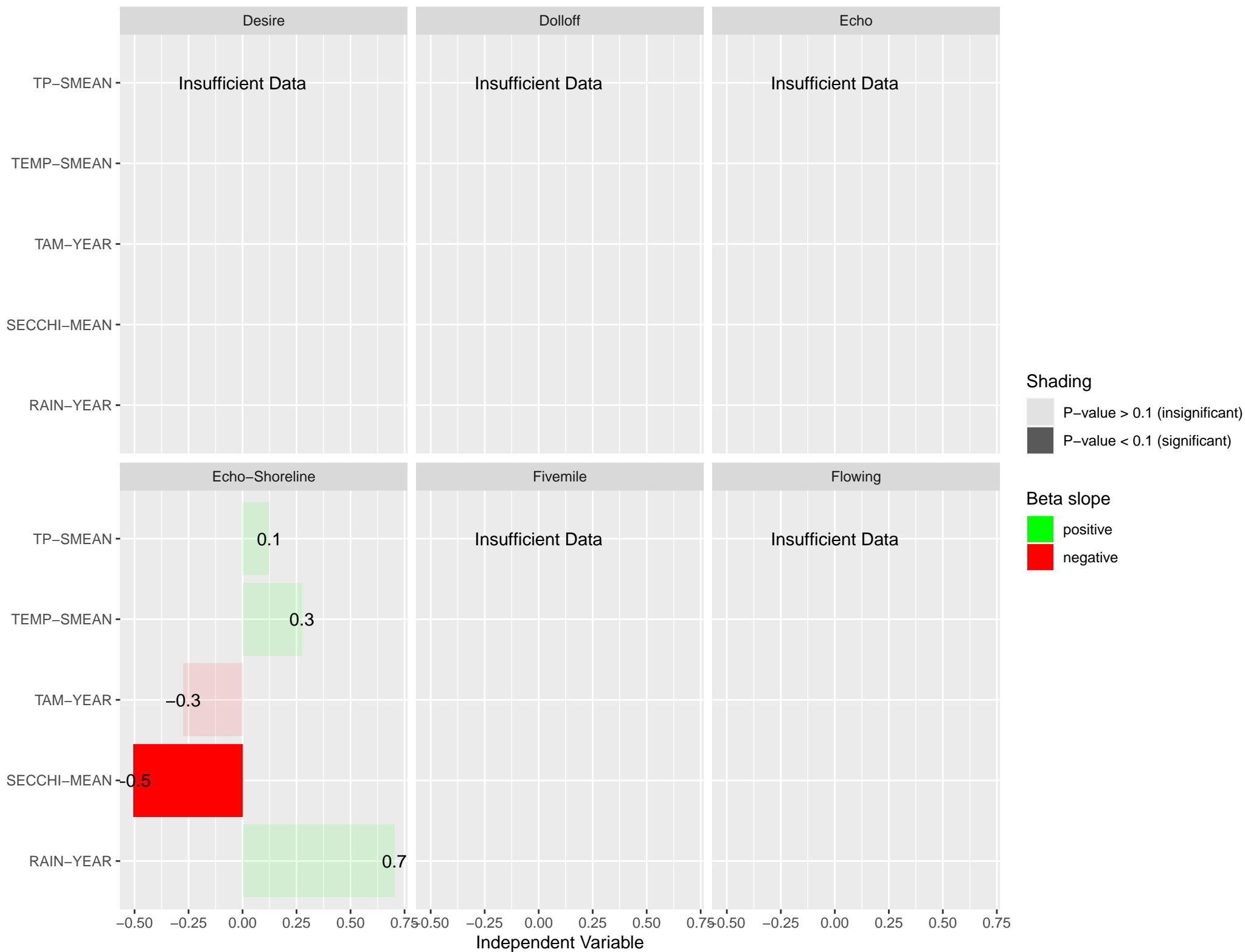
Multiple Regression Results for MC-MAX (Spring data)



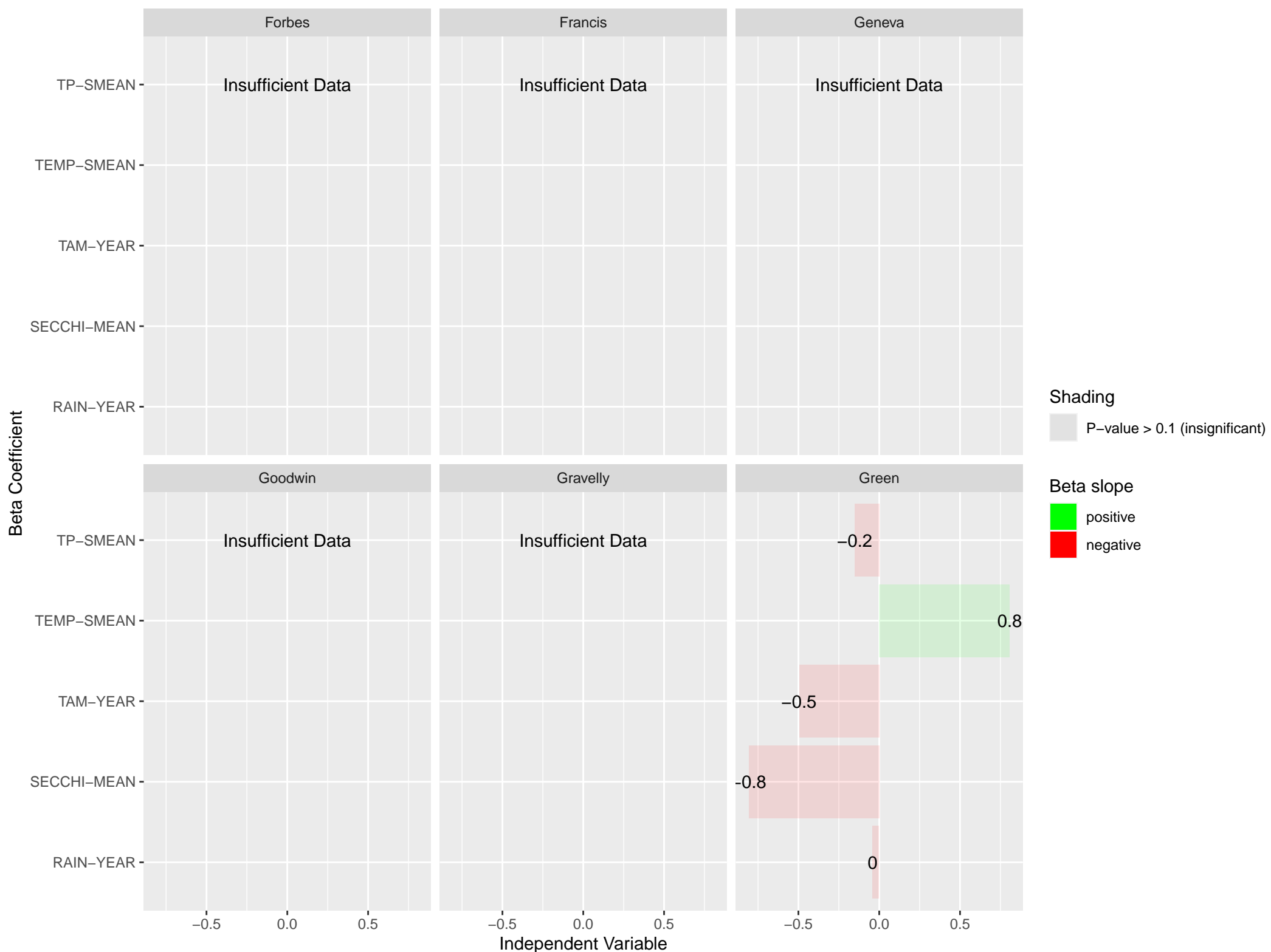
Multiple Regression Results for MC-MAX (Spring data)



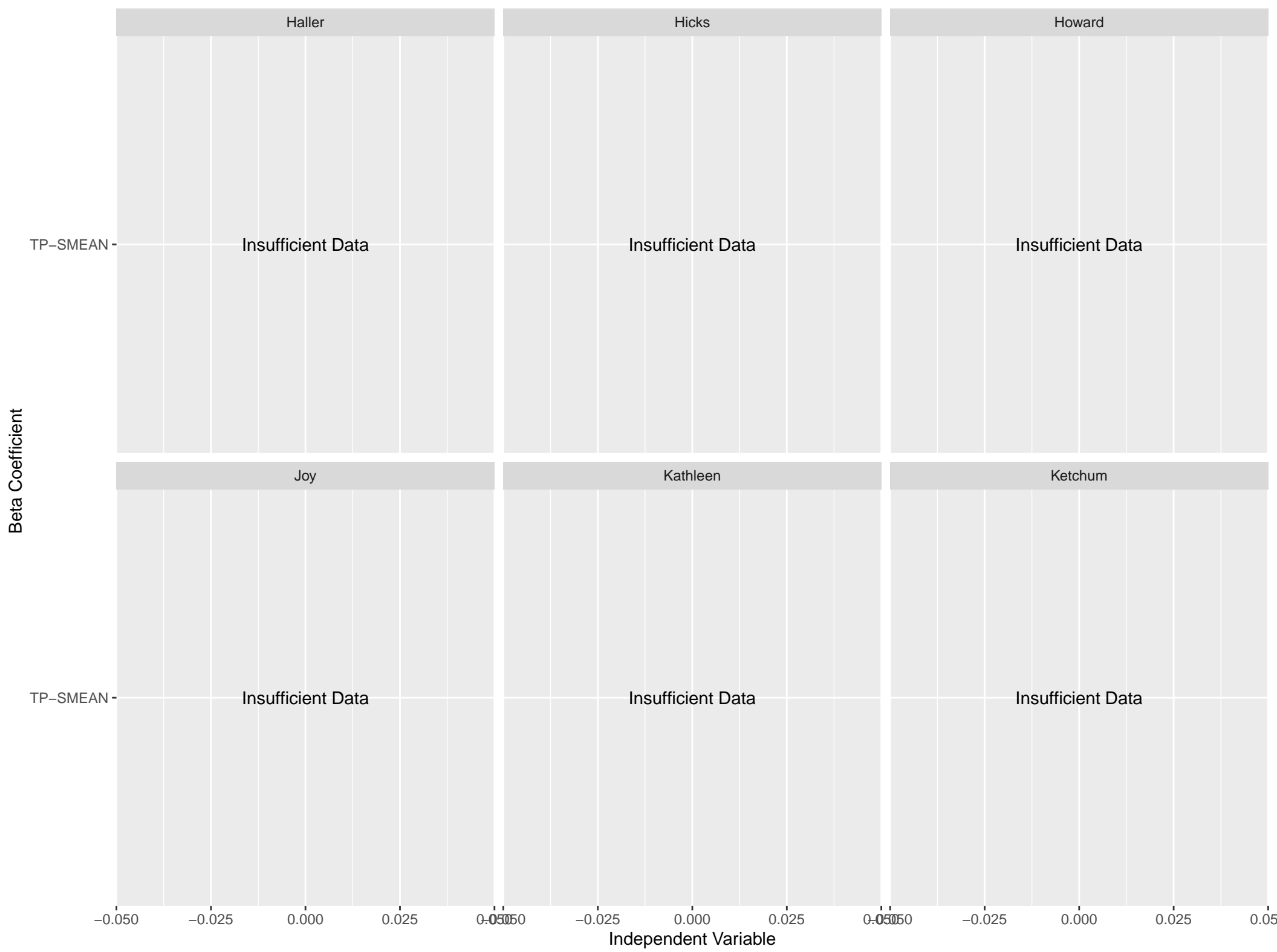
Multiple Regression Results for MC-MAX (Spring data)



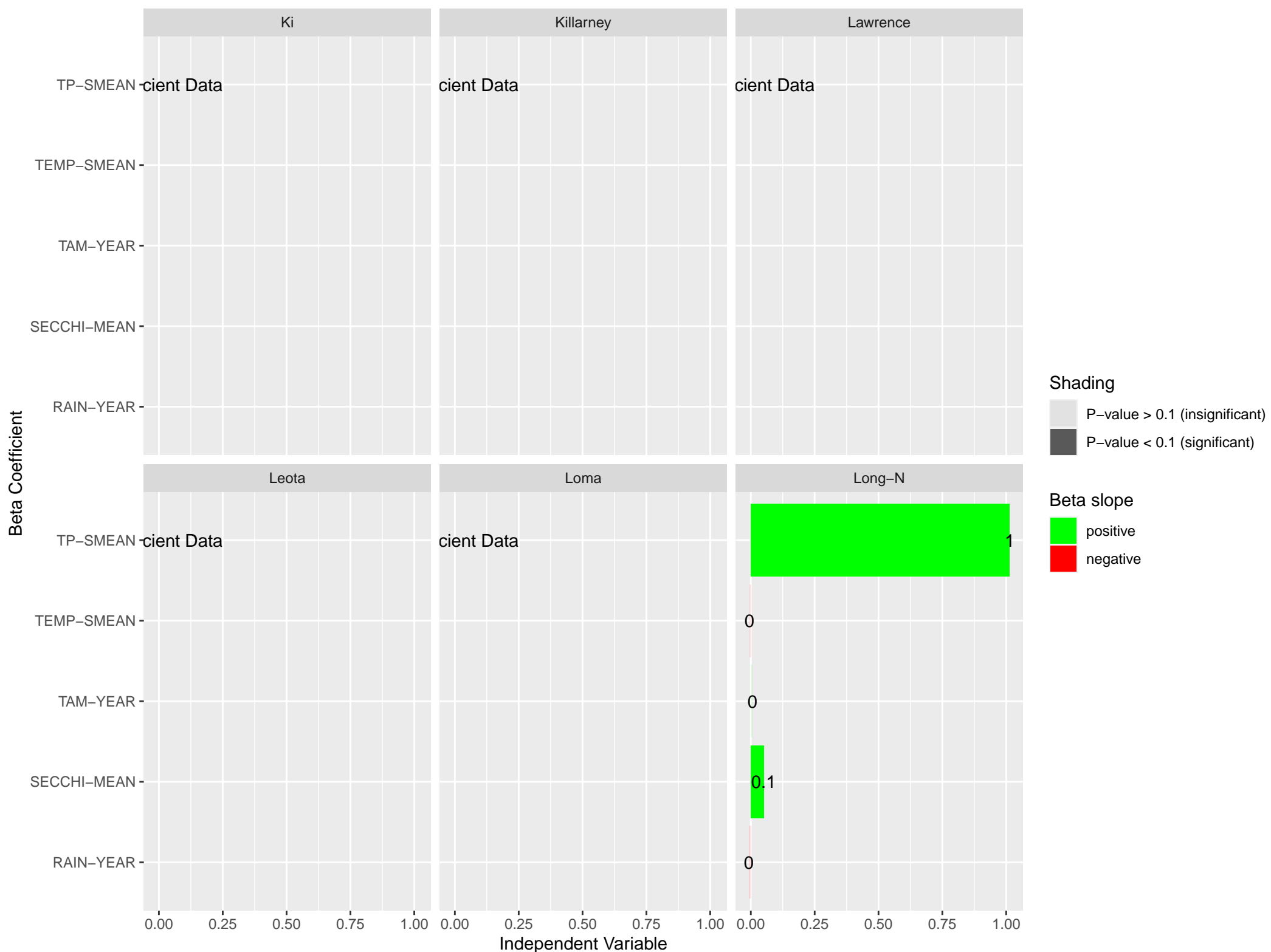
Multiple Regression Results for MC-MAX (Spring data)



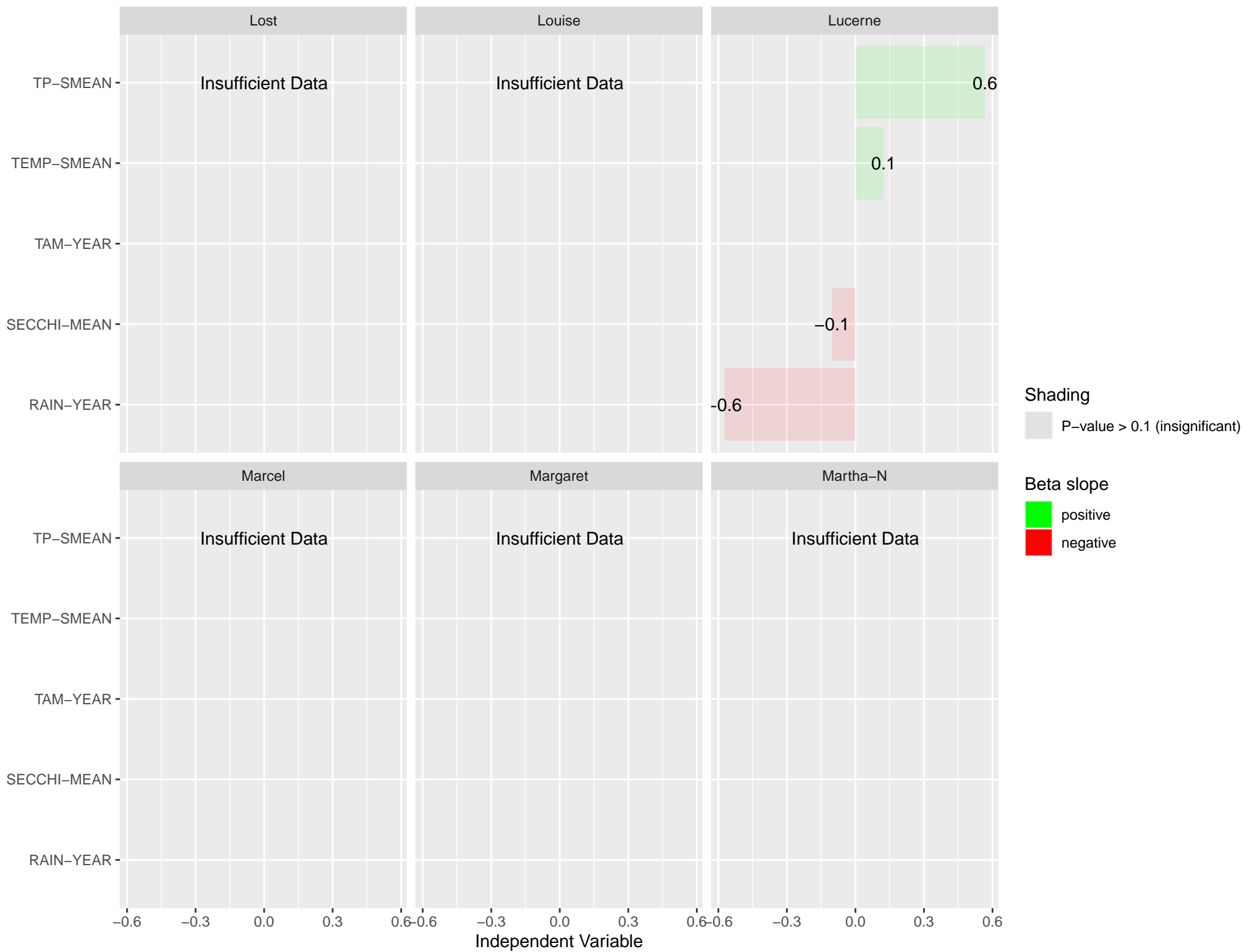
Multiple Regression Results for MC-MAX (Spring data)



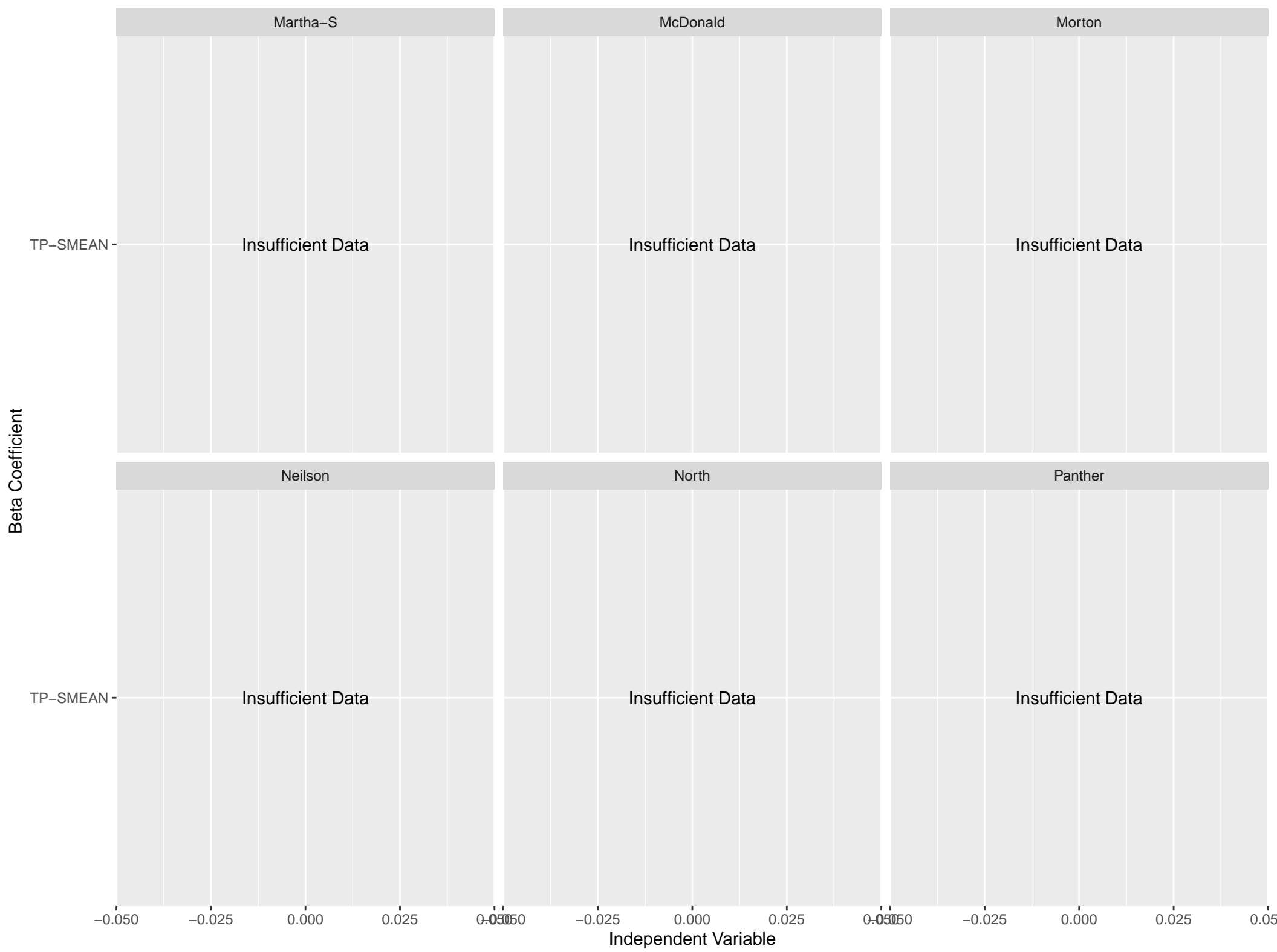
Multiple Regression Results for MC-MAX (Spring data)



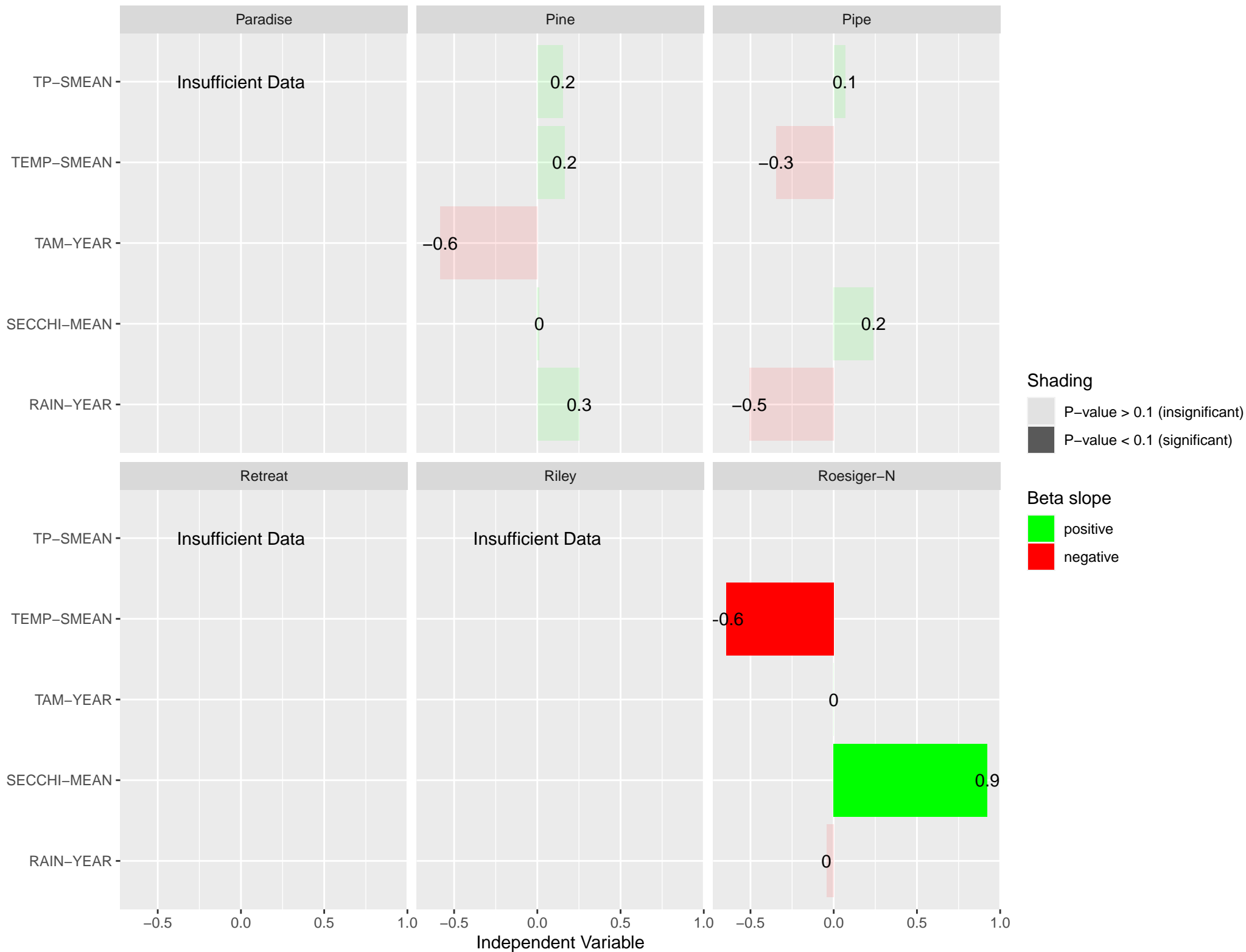
Multiple Regression Results for MC-MAX (Spring data)



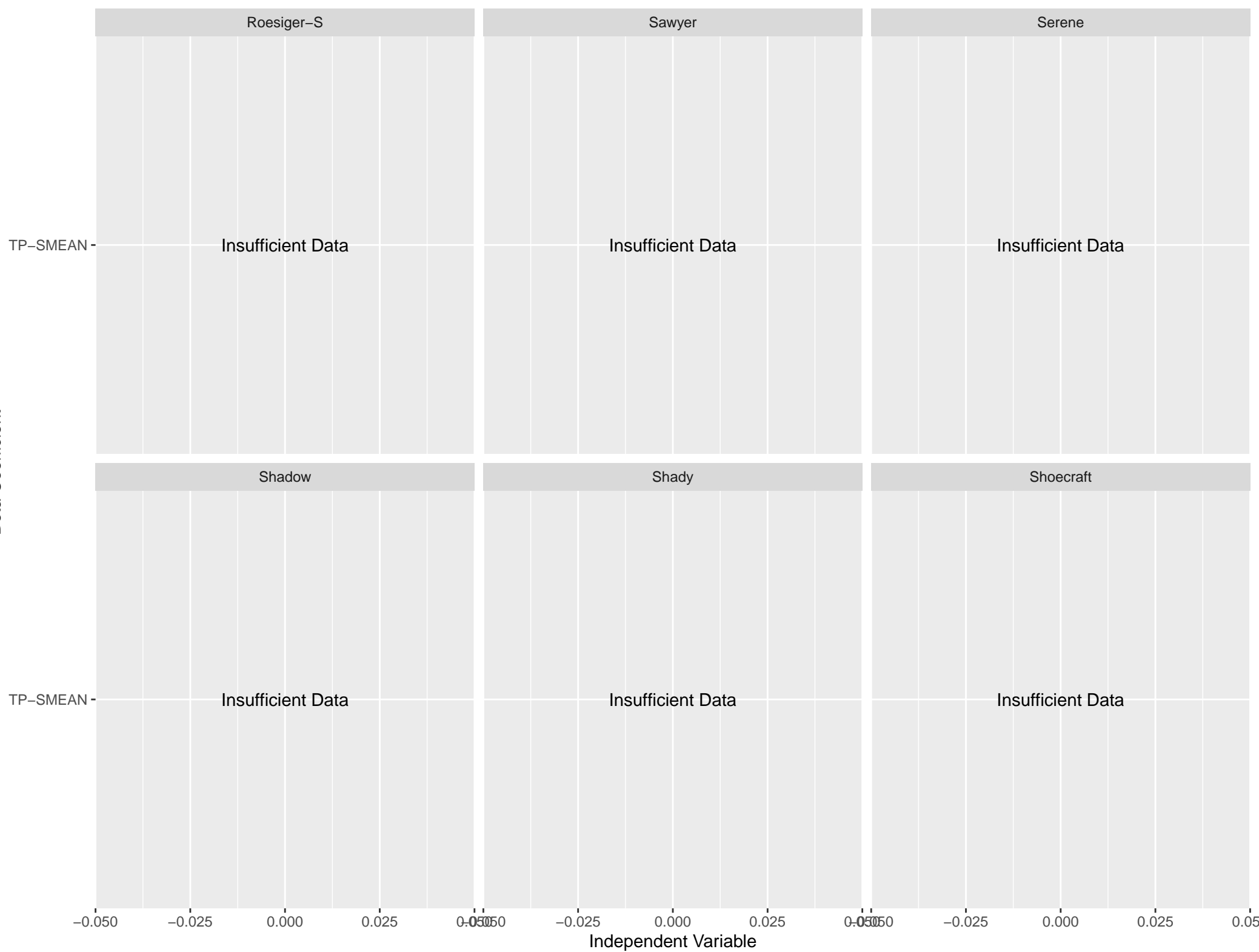
Multiple Regression Results for MC-MAX (Spring data)



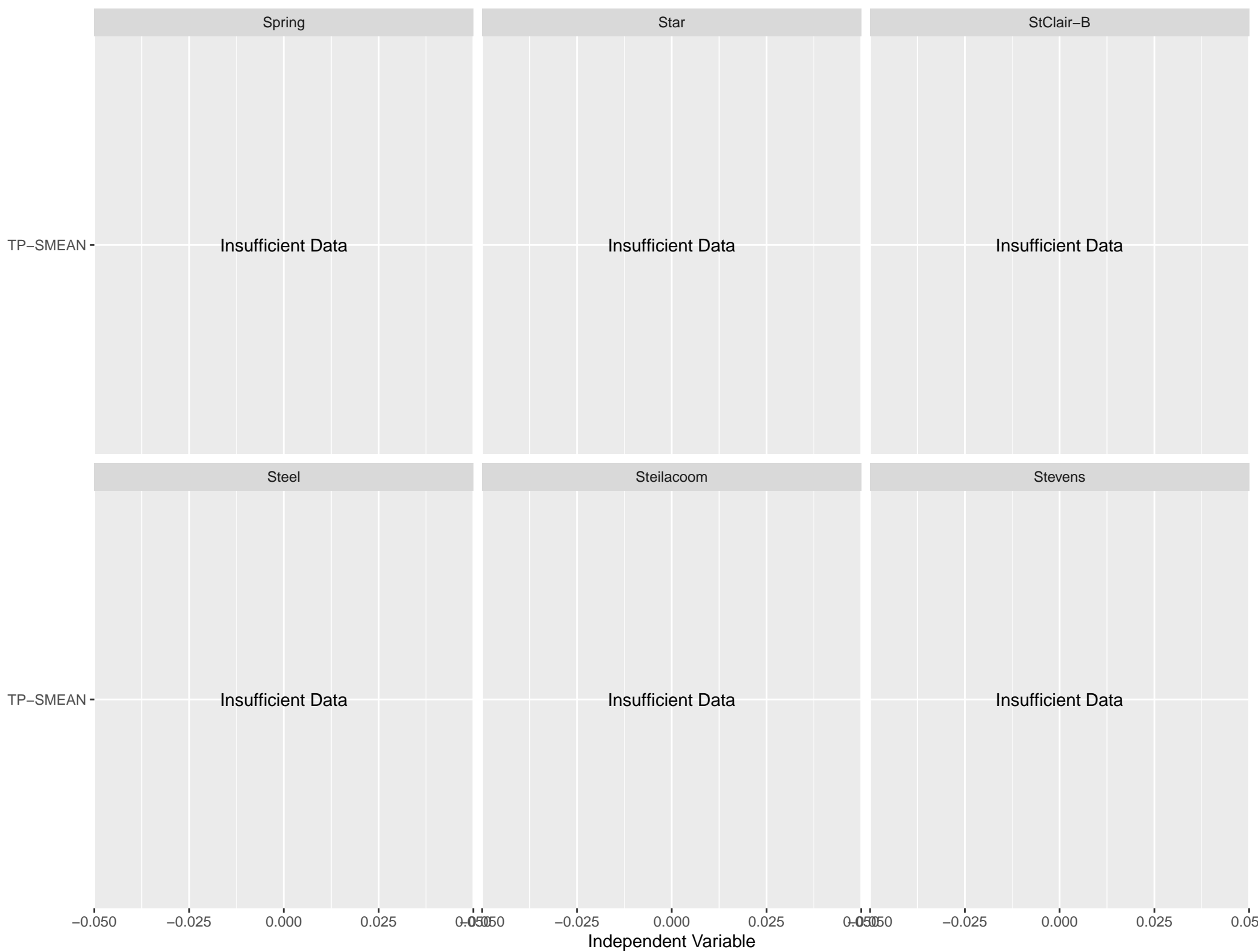
Multiple Regression Results for MC-MAX (Spring data)



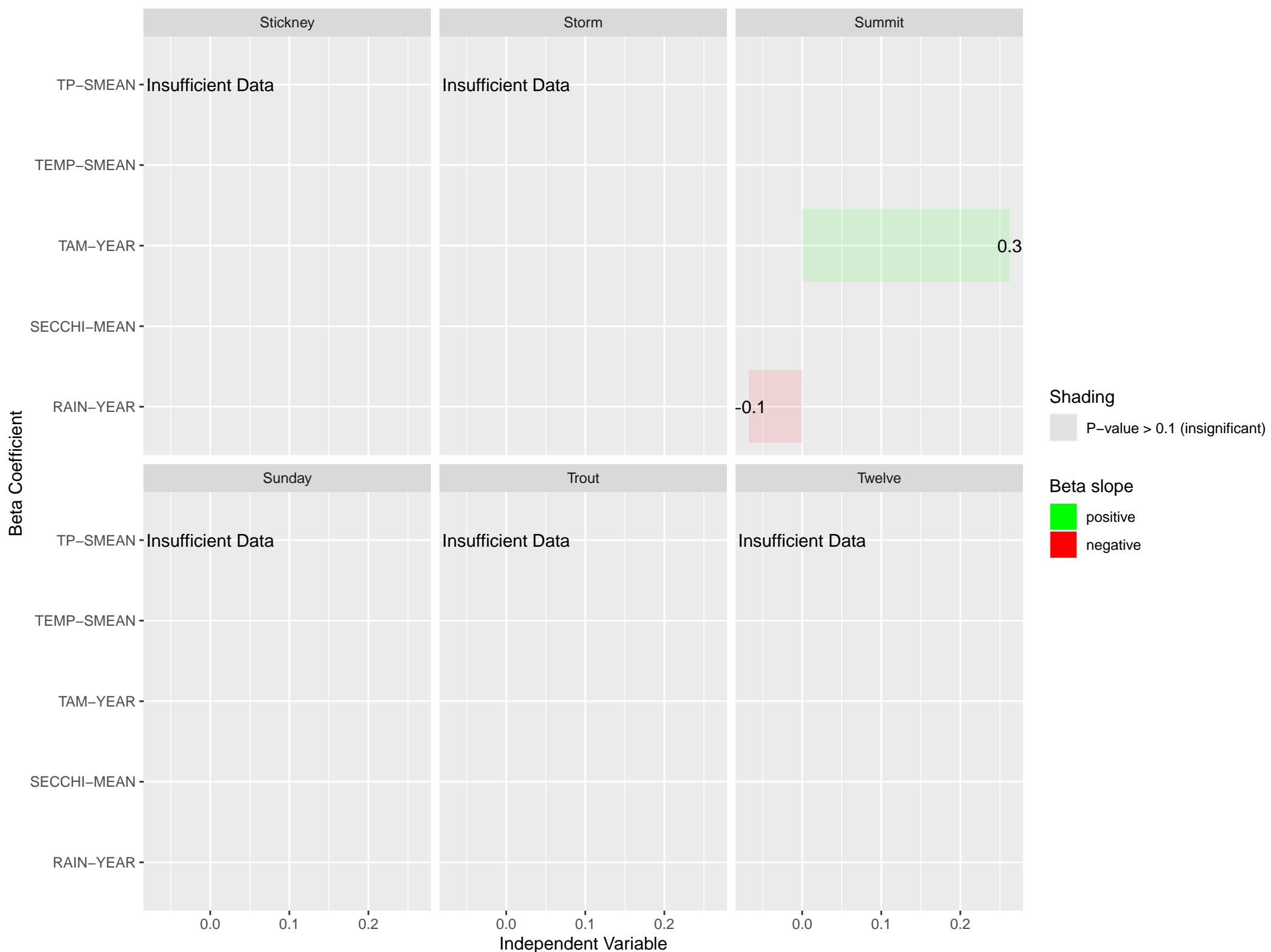
Multiple Regression Results for MC-MAX (Spring data)



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