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Long-Term Influence of Glyphosate Herbicide on Demography and Diversity of Small Mammal Communities in Coastal Coniferous Forest

Abstract

This study tested the hypothesis that (1) abundance and related demographic parameters of small mammal populations, and (2) species diversity of small mammal communities, would be adversely affected in herbicide-treated habitats at 9 and 11 years after treatment in coastal coniferous forest. Study areas were located in south-coastal British Columbia, Canada, in the Coastal Western Hemlock (CWH_{dm}) biogeoclimatic zone where small mammal populations were intensively sampled on paired control and treatment sites. Average density of deer mice (*Peromyscus maniculatus*) during summer periods (1981-1990) was lower in the treatment than control in the immediate post-treatment (PT) period (1982), with comparable numbers in 1983, 1985, and 1990. At the 11-year PT area, deer mouse numbers were similar on control and treatment sites in 1978, 1979, 1980, and 1990. Average density of Oregon voles (*Microtus oregoni*) was higher on treatment than control sites at 9 and 11 years after treatment. Townsend chipmunks (*Eutamias townsendii*) tended to be less abundant on the treatment than control site 9 years PT but were essentially absent from the 11-year PT study area. There was no difference in average density of shrew (*Sorex* spp.) populations between control and treatment sites at either study area. It is likely that post-harvest successional change has more of an impact on small mammal abundance than change induced by a herbicide treatment. Our results suggest that glyphosate herbicide did not adversely affect reproduction, survival, or growth of deer mice and Oregon voles in coastal forest a decade after application. Species richness and diversity of small mammal communities changed little over the decade following treatment. This study is the first investigation on the effects of forest herbicide use on demography and diversity of small mammal communities that extends to a decade.

Introduction

Vegetation management is an important part of forest renewal in the temperate coniferous forests of North America. Silviculture programs must deal with the problem of reducing woody and herbaceous vegetation which competes with desired coniferous trees (McCormack 1985, Walstad and Kuch 1987, Lautenschlager 1990, Newton et al. 1992). Use of herbicides or manual methods as vegetation management tools are usually applied to early successional forest habitats for conifer release. This "release" from competing vegetation has consistently increased growth and survival of crop trees (Walstad and Kuch 1987), although herbicide treatments are consistently superior.

Herbicide related changes in wildlife habitat (food and cover) are a major environmental concern (Lautenschlager 1986, Freedman 1991). Recent studies (Newton et al. 1984, USDA 1984, Atkinson 1985, Feng and Thompson 1990) document that herbicides, properly used for conifer release, pose minimal toxicological hazard for terrestrial verte-

brates. Several studies, reviewed by Lautenschlager (1993), have documented the response of small mammal populations to herbicide-induced habitat alteration in these young coniferous forests. In general, small mammal responses are species specific: some species are unaffected, while some select and others avoid herbicide-treated areas. Although much work has been conducted on the effects of herbicides on small mammals and their habitats, no studies followed small mammal populations and habitat changes for more than 4 years post-treatment (Sullivan 1990a, b). Indeed, valid generalizations about the effects of these treatments on small mammals are limited to one, or two, growing seasons after treatment (Lautenschlager 1993).

There is a paucity of long-term studies on herbicide use in the forest environment. This is particularly relevant to concerns regarding the potential long-term toxicological effects of herbicides on wildlife. Thus, this study was designed to test the hypothesis that (1) abundance and related demographic parameters of small mammal populations, and (2) species diversity of small mammal

communities, would be adversely affected in herbicide-treated habitats at 9 and 11 years after treatment in coastal coniferous forest.

Materials and Methods

Description of Study Areas

This study was located at the University of British Columbia Malcolm Knapp Research Forest near Maple Ridge, B.C., Canada (49°16'N; 122°34'W) on sites in the Coastal Western Hemlock (CWH_{dm}) biogeoclimatic zone (Meidinger and Pojar 1991) between 140 and 400 m elevation. Paired control-treatment sites comprised the 9-year post-treatment (PT) study area. The control site (24.0 ha) was clear-cut harvested in 1973, slash-burned in the fall of 1974, and planted with Douglas-fir (*Pseudotsuga menziesii*) in the spring of 1975. The area was previously covered with a mature (70- to 90-year-old) forest dominated by western hemlock (*Tsuga heterophylla*), Douglas-fir and western red cedar (*Thuja plicata*) (Feller 1977). Cover included well decomposed slash with an abundance of deciduous trees, shrubs and herbaceous vegetation. Associated with the Douglas-fir stand were western hemlock natural regeneration and deciduous tree species such as red alder (*Alnus rubra*) and paper birch (*Betula papyrifera*). Shrub species included salal (*Gaultheria shallon*), willow (*Salix* spp.), vine maple (*Acer circinatum*), black raspberry (*Rubus leucodermis*) and salmonberry (*Rubus spectabilis*). Bracken fern (*Pteridium aquilinum*) and fireweed (*Epilobium angustifolium*) were the dominant herbaceous annual species. The treatment site (23.1 ha) was also clear-cut in 1973 and planted with Douglas-fir in 1975. This area was not burned. The previous forest was also dominated by mature western hemlock, mixed with Douglas-fir and western red cedar. The main cover was well decomposed slash with a similar vegetation composition as the treatment site. Control and treatment sites were separated by approximately 1.2 km which was a sufficient distance to limit small mammal dispersal between the two sites.

Paired control-treatment sites also comprised the 11-year PT study area. The control site was originally an untreated young successional stage reported by Sullivan and Sullivan (1981, 1982), but because

of subsequent silvicultural treatments, this site had to be replaced for the current study. Therefore, a new control site (5.0 ha) clear-cut harvested and slashburned in 1958 and planted with Douglas-fir in 1959 was selected for sampling in 1990. This site was previously occupied by a forest composed of Douglas-fir, western hemlock, and western red cedar which originated after a wildfire in 1868. Because of comparable stand histories, we assumed that this site had a similar vegetative succession to that of the treatment. This site was dominated by Douglas-fir with a minor component of western hemlock, western red cedar, red alder, and cascara (*Rhamnus purshiana*) in the overstory. The understory was composed of salmonberry and sword fern (*Polystichum munitum*) with some salal, red huckleberry (*Vaccinium parvifolium*), thimbleberry (*Rubus parviflorus*), trailing blackberry (*Rubus ursinus*), deer fern (*Blechnum spicant*) and spiny woodfern (*Dryopteris assimilis*).

The treatment site (15.0 ha) was harvested and burned in 1959. A Douglas-fir plantation was established in 1960. This site was previously occupied by the same forest composition which originated from the 1868 wildfire. Dominant vegetation on this site in 1979 was Douglas-fir, vine maple, red alder, bitter cherry (*Prunus emarginata*), and bigleaf maple (*A. macrophyllum*), with an understory of salmonberry, sword fern, and elderberry (*Sambucus racemosa*). In 1990, this site had a similar species composition to that of the control. Control and treatment sites were separated by approximately 2.0 km.

Application of Herbicide

The 9-year treatment area (14.2 ha of the 23.1-ha site) was aerially sprayed with Roundup® (or Vision®) herbicide¹ at the rate of 3.0 kg/ha of active ingredient on 18 June 1982.

The 11-year treatment area (4.9 ha of the 15.0-ha site) was aerially sprayed with Roundup® at the rate of 2.2 kg/ha of active ingredient on 12 September 1979.

Small Mammal Populations

One sampling grid (1 ha) was located in each of the 2 control and 2 treatment sites. On each grid, 49 (7 x 7) trap stations were located at 14.3-m intervals and one Longworth live-trap was placed within a 2-m radius of each station. Traps were baited with peanut butter, whole oats, and coarse

¹Commercial formulations (trademarks of Monsanto Company) containing glyphosate, 356 g/L present as isopropylamine salt

brown cotton was supplied as bedding. Traps were set on day 1, checked on days 2 and 3, and then locked open between trapping periods. The four grids were live-trapped at 3-week intervals from May to November 1990, which was 9 and 11 years after treatment, respectively, for the two study areas. For the 9-year PT study area, the sampling grids were originally live-trapped from April 1981 to September 1983 and from April to October 1985. For the 11-year PT study area, the sampling grids were live-trapped from July to November 1978, May to November 1979, and April to November 1980.

All small mammals (except shrews, *Sorex* spp., American shrew-moles, *Neurotrichus gibbsii*, and weasels *Mustela* spp.) captured were ear-tagged with serially numbered fingerling fish tags, sexed, reproductive condition noted, and weighed on Pesola spring balances. Reproductive performance was noted by palpation of male testes and mammarys of the females (Krebs et al. 1969). Small mammals were released on the grids immediately after processing. The major small mammal species captured included the deer mouse (*Peromyscus maniculatus*), and Oregon vole (*Microtus oregoni*). Other less common species sampled included the Townsend chipmunk (*Tamias townsendii*), southern red-backed vole (*Clethrionomys gapperi*), long-tailed vole (*M. longicaudus*), and Pacific jumping mouse (*Zapus trinotatus*).

Body weight was used as an index of age. For the numerically dominant deer mice and Oregon voles, animals were classified as juvenile or adult by weight (deer mice: juv = 0-16 g, adult \geq 17 g; voles: juv = 0-20 g, adult \geq 21 g) after Sullivan (1990b). Juveniles were considered to be young animals recruited during the study. Recruits were defined as new animals that entered the population through reproduction and immigration.

Demographic Analysis

Minimum number of animals known to be alive (MNA) (Krebs 1966) was calculated for populations of deer mice and Oregon voles. MNA was selected (Hilborn et al. 1976) because the generally preferred Jolly-Seber probabilistic estimator (Seber 1982) became unreliable and impossible to calculate for voles and chipmunks with low recaptures of previously marked animals (Krebs et al. 1986). Boonstra (1985) found that the MNA and Jolly-Seber techniques provided similar estimates

of small mammal population size under field conditions when trappability exceeded 50%. The total number of individuals captured was used to compare control and treatment populations of southern red-backed voles, long-tailed voles, Pacific jumping mice, and American shrew-moles; and MNA was calculated for Townsend chipmunks.

Minimum survival rates for all males and females in control and treatment populations of deer mice and Oregon voles were summed over each summer period with an individual animal being tallied each time it was captured. Early juvenile survival, defined as the percentage of observed juveniles/expected number of juveniles, was calculated for deer mice and Oregon voles. Expected number of juveniles was the product of the number of successful pregnancies (based on consecutive captures of lactating females) and the average number of juveniles per litter as recorded in the literature: 4.5/litter for the deer mouse (Sheppe 1963, Sadleir 1974); 3.2/litter for the Oregon vole (Gashwiler 1972).

Mean body weights of male deer mice and Oregon voles during summer periods were used as an index of condition within control and treatment populations. Female weights were omitted because these data are complicated by undetected pregnancies, and hence tend to be more variable than those for males.

Species Richness and Diversity

Species richness was measured by the total number of species sampled (Krebs 1989). Species diversity was measured by Simpson's index of diversity (Simpson 1949) which is sensitive to changes in the more abundant species. The Shannon-Wiener index of diversity (Pielou 1966) was also used because it is sensitive to changes in the rare species in a community sample. These diversity measures were calculated using MNA values for the three common species and number of individuals captured for the less abundant species in a given sampling period and represented by an average value for each year.

Statistical Analysis

Average density of animals per ha and 95% confidence limits were calculated for the populations of deer mice and Oregon voles in all pre- and post-treatment periods for each study area. A paired

sample *t*-test was used to compare average density of shrew populations between control and treatment sites for the 9- and 11-year PT study areas. A chi-square analysis was used to compare proportion of breeding animals, observed versus expected number of juveniles, and total minimum survival rates for summer periods between paired control and treatment populations. Because some samples were not completely independent, these comparisons were used as an indication of the degree of difference between sets of data. Comparison of body weights and average diversity values between control and treatment populations was done with average values and 95% confidence limits. In all analyses, the level of significance was at least $P = 0.05$.

Results

Abundance

Average density of deer mice during summer periods from 1981 to 1990 indicated a lower abundance in the treatment than control in the immediate post-treatment period (1982), with comparable numbers in 1983, 1985, and 1990 (Fig. 1A). Average density of deer mice during summer sampling periods on 11-year sites was similar between control and pre-treatment sites in 1978, higher on the pre-treatment than control in 1979, and similar in the post-treatment years of 1980 and 1990 on control and treatment sites (Fig. 1B).

Average density of Oregon voles was similar between control and treatment sites in the 9-year PT study area up to 1990 (Fig. 2A). As in the 9-year area, the treatment population of voles was higher than the control at the 11-year area in 1990 (Fig. 2B). Average density of voles was higher on the control than treatment site in 1978 to 1980 at the 11-year area.

Average densities of shrews per trapping period provided a relative abundance of this insectivore on control and treatment areas, despite a high mortality because of the overnight trapping technique. Before treatment there were 2.1 times as many shrews per ha on the treatment than control site at the 9-year PT study area in 1981 (Table 1). There was, however, no statistical difference ($t = -1.46$, $P = 0.21$) in average density of shrews between control and treatment sites over the period of this study. At the 11-year PT study area, average densities of shrews were similar between control and treatment sites before treatment (1978, 1979) and

during the first post-treatment year (1980). There were, however, 1.9 times as many shrews on the treatment than control site in 1990 (Table 1). Overall, there was no difference ($t = -1.32$, $P = 0.30$) in average density of shrews during the course of this study.

Details of changes in abundance of these two rodent species and *Sorex* spp. prior to 1990 are reported in Sullivan and Sullivan (1982) and Sullivan (1990a).

Reproduction

There were no differences in proportion of breeding males between control and treatment populations of deer mice at either of the 9- or 11-year PT study areas in 1990. There were significantly more reproductive female deer mice in the control than treatment population in 1990 for the 9-year PT, but no difference for the 11-year PT study area. There were no significant differences in proportion of breeding Oregon voles between control and treatment populations in 1990 at either study area.

The control population of deer mice had twice as many successful pregnancies as the treatment population in 1990 at the 9-year PT study area, with the same number (19) at the 11-year PT study area (Table 2). However, percentage recruitment of juvenile deer mice in control and treatment populations was similar at both areas. The number of successful pregnancies for Oregon voles tended to be higher in treatment than control populations. This was reflected in the significantly higher (3.2 times) percentage of recruits on treatment than control sites at the 9-year PT study area, and relatively higher (2.2 times) numbers treatment vs. control at the 11-year PT study area (Table 2). Details of this measure of reproductive success and early juvenile survival on these areas prior to 1990 are reported in Sullivan and Sullivan (1981) and Sullivan (1990b).

Survival

Total survival of deer mice and Oregon voles during summer periods showed no consistent differences between control and treatment populations in 1990 at the 9-year nor 11-year PT study areas.

Body Weight

Populations of male *P. maniculatus* had similar body weights during summer 1990 at the 9-year PT study

Peromyscus maniculatus

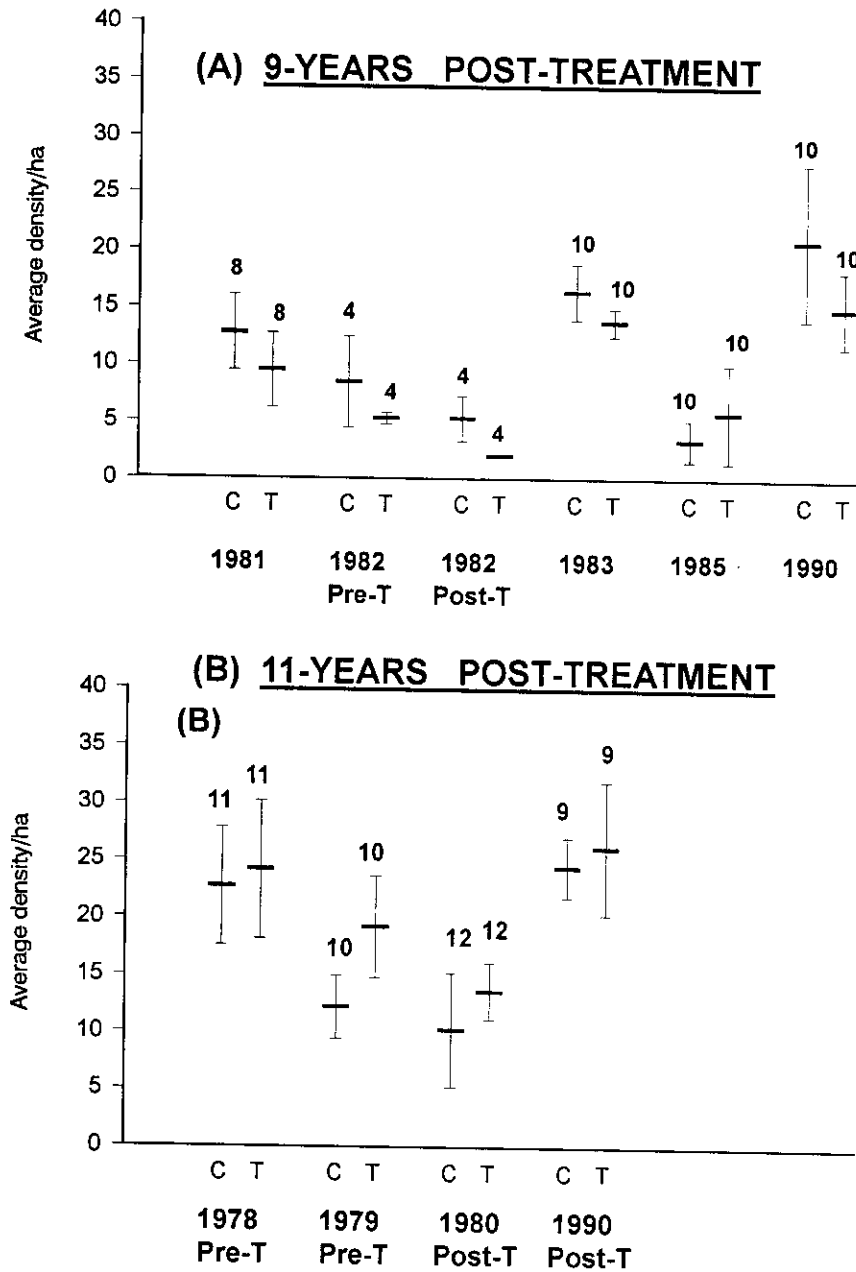


Figure 1. Average population density with 95% confidence limits of *Peromyscus maniculatus* on control and treatment sites at the (A) 9-year PT study area, and (B) the 11-year PT study area. Sample size (number of trapping periods) above upper limit. C = control; T = treatment.

Microtus oregoni

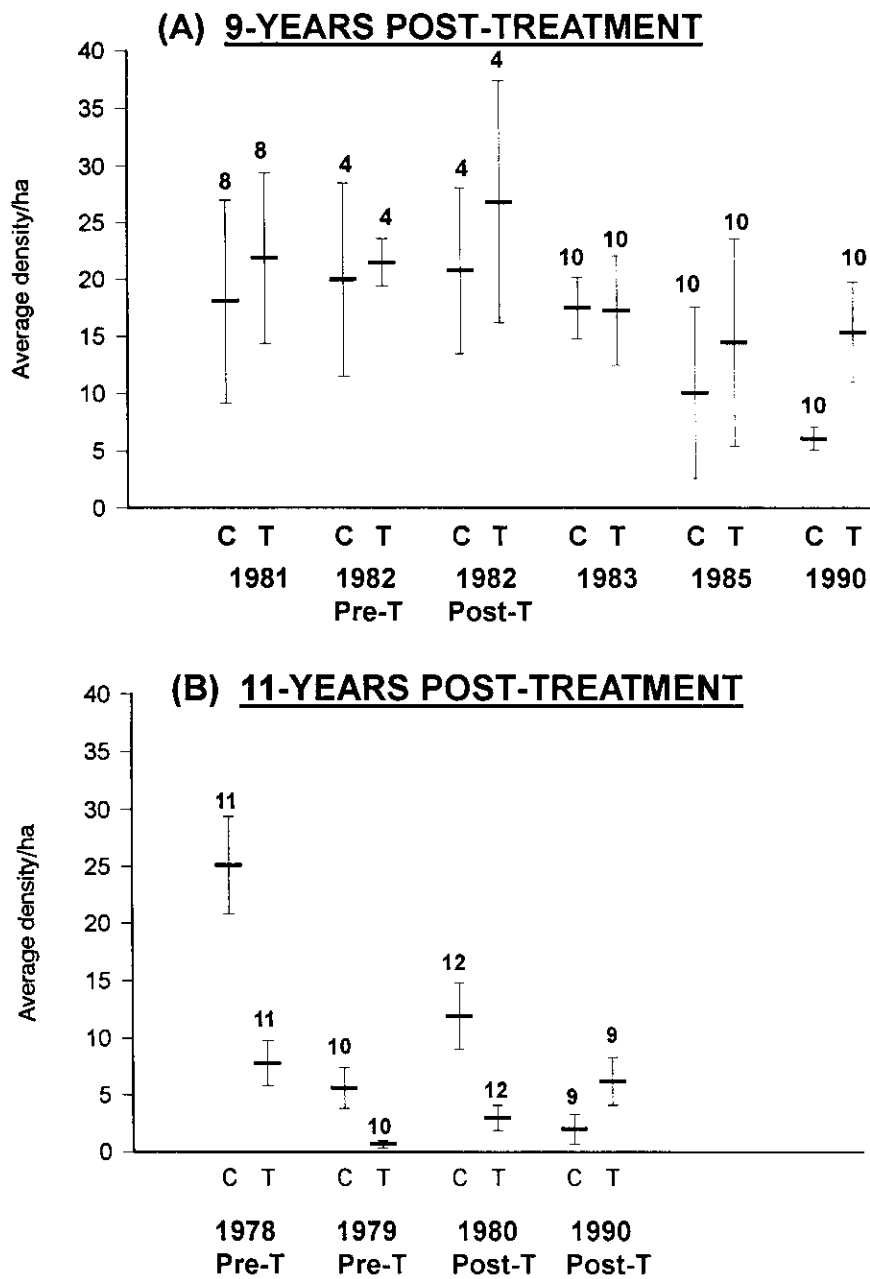


Figure 2. Average population density with 95% confidence limits of *Microtus oregoni* on control and treatment sites at the (A) 9-year PT study area, and (B) the 11-year PT study area. Sample size (number of trapping periods) above upper limit. C = control; T = treatment.

TABLE 1. Average density of individual shrews (*Sorex* spp.) captured per ha on control and treatment sites in the 9-year and 11-year post-treatment (PT) study areas.

Study area and year	Number of sampling periods	Control	Treatment
<u>9-year PT</u>			
1981 pre-T	8	2.6	5.5
1982 pre-T	4	3.0	2.5
1982 post-T	4	3.5	4.8
1983 post-T	10	1.3	1.9
1985 post-T	10	2.0	1.9
1990 post-T	10	1.5	1.7
<u>11-year PT</u>			
1978 pre-T	11	1.6	2.8
1979 pre-T	10	3.3	3.5
1980 post-T	12	3.1	2.6
1990 post-T	9	1.9	3.7

area with average values and 95% confidence limits in the control (15.8 g; 15.2-16.5) and treatment (16.2 g; 15.6-16.9), respectively. *M. oregoni* had heavier male animals in the control (22.6 g; 21.8-23.4) than the treatment (20.5 g; 19.6-21.5) in 1990. However, adult male voles tended to be heavier in the treatment (24.1 g; 23.5-24.7) than control (23.2 g; 22.8-23.5).

At the 11-year PT study area, populations of male deer mice in 1990 had similar average body weights in the control (15.6 g; 15.0-16.3) and treatment (15.0 g; 14.5-15.4), respectively. Populations of *M. oregoni* were also similar in average body

weight in 1990 in the control (21.3 g; 17.1-25.6) and treatment (17.7 g; 15.5-19.8), respectively.

Species Richness and Diversity

Species richness of the small mammal communities was similar between control and treatment sites for all periods at the 9-year PT study area (Fig. 3A). Both measures of species diversity also followed this pattern with average values ranging from 0.47 to 0.67 for Simpson's Index (Fig. 3B) and from 1.34 to 1.74 for Shannon-Wiener (Fig. 3C). At the 11-year PT study area, species richness was similar between control and treatment sites but diversity declined in 1979 on the pre-treatment, and in 1980 on the post-treatment, compared with the control site (Fig. 4A). This decline coincided with the drop in Oregon vole abundance (Fig. 2B). Species diversity tended to be higher on the treatment than control in 1990 (Figs. 4B and C).

Discussion

Experimental Design

This study investigated the 9- and 11-year responses of small mammal populations to herbicide use for conifer release in young (7-year-old) and older (20-year-old) Douglas-fir plantations, respectively. Based on the classification of forest successional stages in western North America (Thomas et al. 1979), over the decade since treatment, the 7-year-old plantation changed successionally from the shrub-seedling stage to pole-sapling (16 years old) and the 20-year-old plantation changed from the pole-sapling to young (31 years old) stand stage.

TABLE 2. Successful pregnancies for *Peromyscus maniculatus* and *Microtus oregoni*, and observed and expected numbers of juveniles recruited into control and treatment populations during 1990 for the 9-year and 11-year post-treatment (PT) study areas. Percentage of juveniles (observed/expected) is given in parentheses.

	<i>P. maniculatus</i>		<i>M. oregoni</i>	
	Control	Treatment	Control	Treatment
<u>9-year PT</u>				
No. of successful pregnancies	12	6	7	10
Expected no. of juveniles	54.0	27.0	22.4	32.0
Observed no. of juveniles	40 (74.1)	26 (96.3)	10 (44.6) ^a	45 (140.6) ^a
<u>11-year PT</u>				
No. of successful pregnancies	19	19	4	7
Expected no. of juveniles	85.5	85.5	12.8	22.4
Observed no. of juveniles	39 (45.6)	44 (51.5)	5 (39.1)	19 (84.8)

a-a, $P < 0.01$. Values followed by same letter are significantly different by Chi-square.

9-YEARS POST-TREATMENT

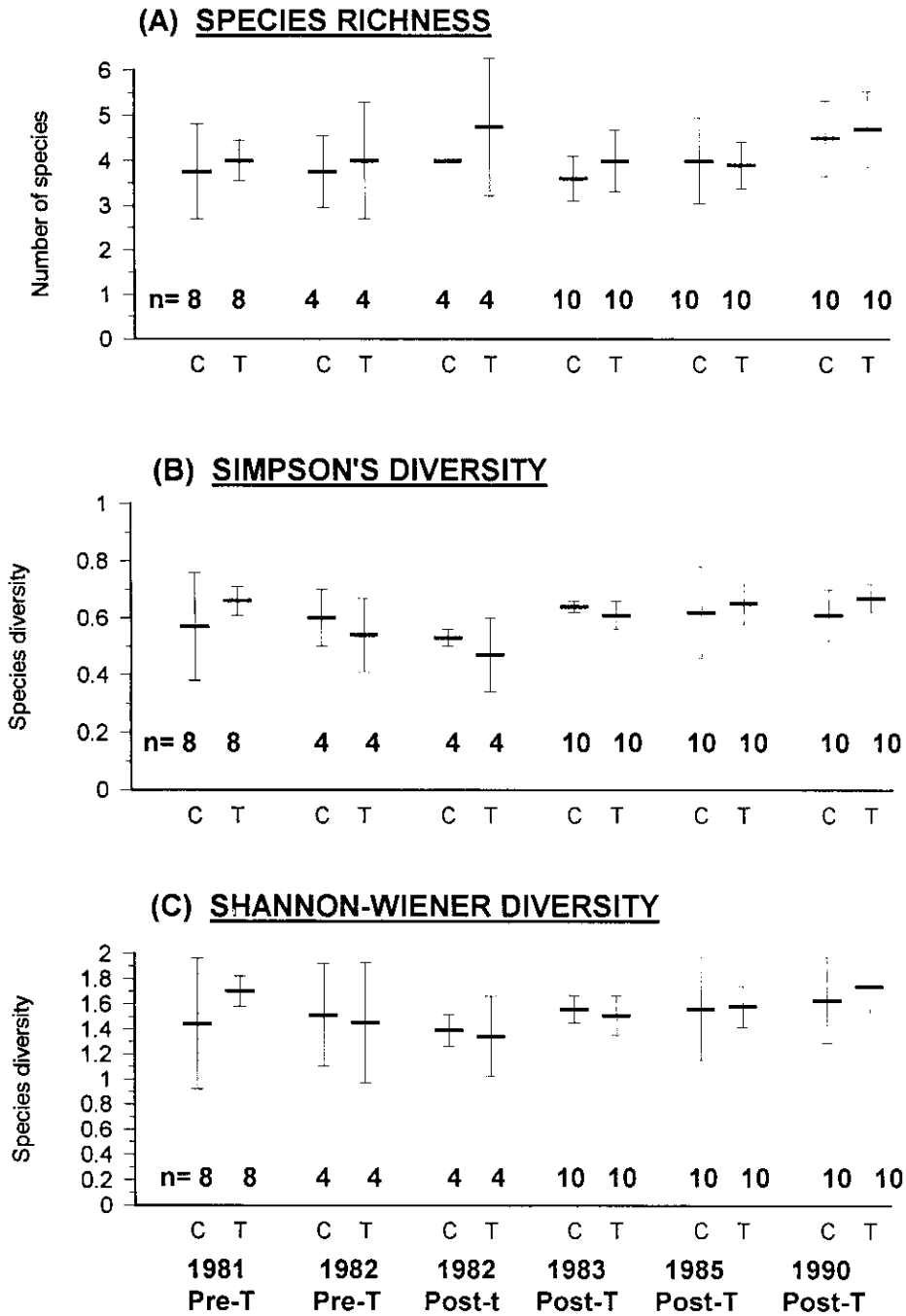


Figure 3. Species richness (A), Simpson's diversity (B), and Shannon-Wiener diversity (C) for small mammal communities on control and treatment sites at the 9-year PT study area. C = control; T = treatment. n = number of trapping periods.

11-YEARS POST-TREATMENT

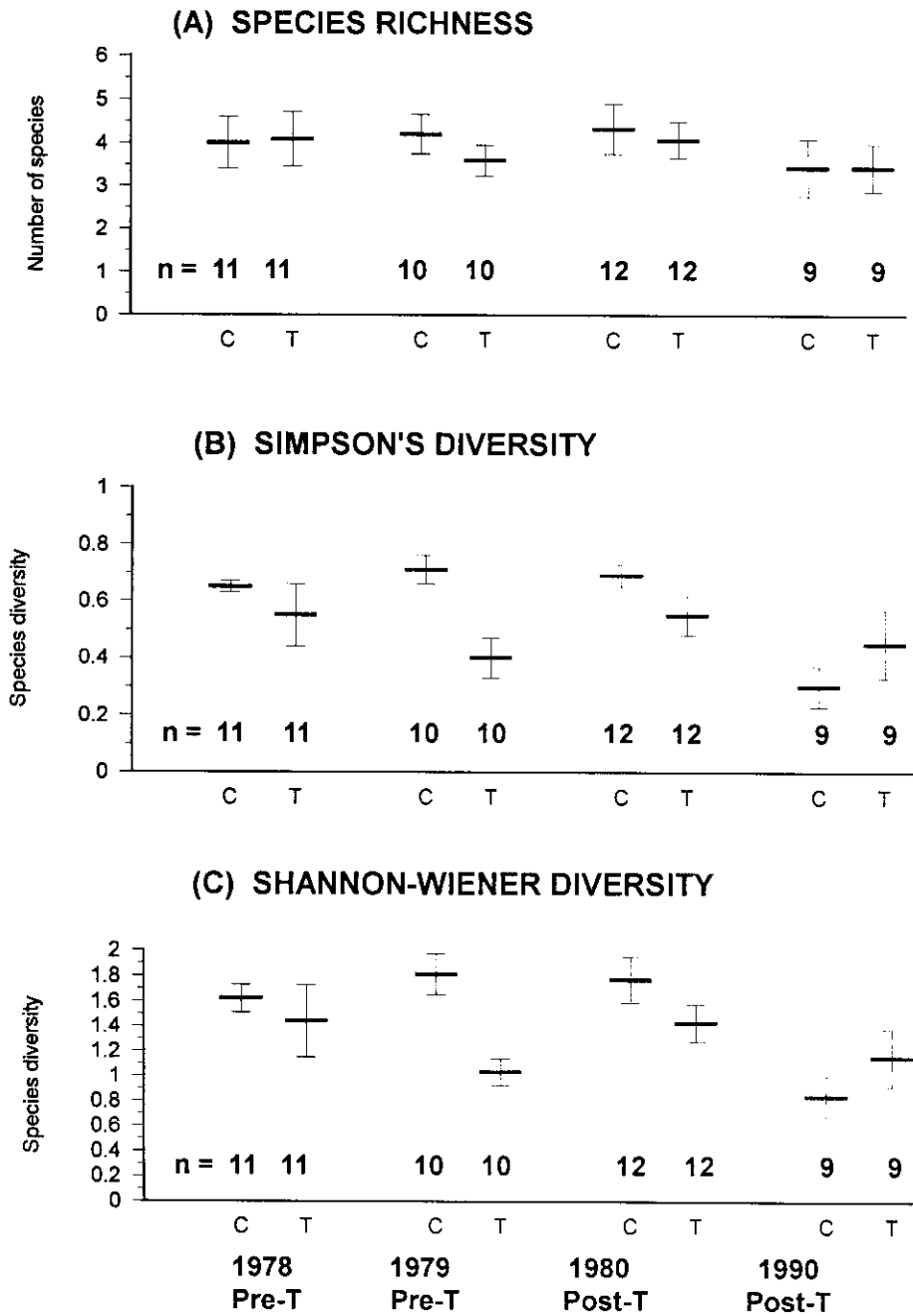


Figure 4. Species richness (A), Simpson's diversity (B), and Shannon-Wiener diversity (C) for small mammal communities on control and treatment sites at the 11-year PT study area. C = control; T = treatment. n = number of trapping periods.

This difference in successional stage precludes the classification of these control-treatment pairs as true replicates (Hurlbert 1984) and our results should be interpreted accordingly. Although we did not have similarity of sites and timing of treatments for adequate replication, pre-treatment sampling was conducted at both study areas and long-term response data were collected. Thus, two of the three requirements were met for providing accuracy and predictive power in forestry-wildlife studies (Lautenschlager 1993).

The 9-year PT study continued on the same control and treatment sites (1981 to 1985) initially reported by Sullivan (1990a, b) using exactly the same grid positions and methods of intensive sampling of the small mammal communities. In the 11-year PT study, the control site (1978 to 1980) had to be changed from that reported by Sullivan and Sullivan (1981, 1982) to a 30-year-old Douglas-fir stand with a similar history to that of the treatment. Identical sampling methodology (and grid position for the treatment) was also used for this study area. Again, however, our results must be viewed within the context of this confounding factor of a "new" control.

Another difference between our two study areas was the June and September applications of herbicide. The June application provided pre-treatment and post-treatment periods within the summer of 1982 since there was clearly a dramatic change in habitat during the growing season (Sullivan 1990a). The mid-September application was at the end of the growing season and herbicide-induced leaf-fall could not be distinguished from that occurring naturally (Sullivan and Sullivan 1982). Thus, both summers of 1978 and 1979 were considered pre-treatment years for the 11-year PT study.

Successional Stage and Herbicide Treatment

Although not documented by data on habitat structure, our control and treatment areas changed dramatically as vegetative succession occurred over the post-treatment decade. Most herbicide-induced changes in herbaceous vegetation persist for only 2-3 years, whereas impacts on shrub species may be considerably longer (Lautenschlager 1990, Newton et al. 1992, Freedman et al. 1993, Sullivan et al. 1996a). Habitat alteration from these herbicide treatments appeared to have little effect on the distribution and abundance of small mammal popu-

lations in the immediate post-treatment years (Figs. 1 and 2; Sullivan and Sullivan 1982, Sullivan 1990a). Other studies in the Pacific Northwest have reported that the application of several herbicides altered the species composition of small mammal communities in Oregon one year after treatment (Black and Hooven 1974, 1979). Similar results were obtained for glyphosate herbicide in the first year after treatment with a return to prespray conditions in the second year (Anthony and Morrison 1985). Cole et al. (1996) reported little change in small mammal populations after herbicide treatment in hardwood conversion areas. Similarly, Runciman and Sullivan (1996) found no significant effects of manual cutting or cut-stump herbicide treatment on small mammal populations two years post-treatment.

It is likely that post-harvest successional change in vegetation has more of an impact on small mammals than that induced by a herbicide treatment. Abundance of deer mice was generally similar on control and treatment areas over the post-treatment decade in the transition from shrub-seedling to pole-sapling to young stand stages. This is not surprising as *P. maniculatus* occupies many different habitats ranging from open fields to old growth forests (Baker 1968).

The higher abundance of Oregon voles on the treatment than control at 9-11 years after herbicide application suggests that openings in the plantations encouraged development of understory herb and shrub species that persisted through time. In 1990, *M. oregoni* populations were, on average, 2.5-3.1 times higher in the pole-sapling (9-year PT) than young (11-year PT) stand. This difference in vole numbers was also evident between control (shrub-seedling) and treatment (pole-sapling) sites in 1978-1980 (Fig. 4B, Sullivan and Sullivan 1982). The Oregon vole occurs primarily in early successional stages after logging as well as in the edges of timber and abandoned brushland (Gashwiler 1970, 1972, Hooven and Black 1976, Sullivan 1980, Sullivan and Krebs 1981). Because canopy closure in our 30-year-old plantations averaged 90% (P. Sanders, pers. comm.), it is not surprising that Oregon vole numbers were low. In addition, Townsend's chipmunks were essentially absent from these stands, since they prefer high quality habitat associated with early successional stages after harvesting (Tevis 1956, Anthony and Morrison 1985), mature-old growth forests (Gashwiler 1959, Rosenberg and Anthony 1993), and riparian areas in second-growth coniferous forests (Anthony et

al. 1987). Where the canopy is opened by stand thinning or conifer release treatments, understory development can provide habitat for various early successional species.

Demography and Diversity

As discussed by Newton et al. (1984), it is likely that small mammals do have some glyphosate residues within their body systems during the post-treatment period. What, then, are the potential long-term (10-year) effects of these residues on demographic attributes of small mammals such as the deer mouse and Oregon vole? Our results suggest that this forest herbicide did not adversely affect reproduction, survival, or growth of animals in treatment areas a decade after application. These results are consistent with earlier studies 4 years post-treatment in coastal coniferous forest (Sullivan 1990b) and 5-years post-treatment in sub-boreal spruce forest (Sullivan et al., unpubl. manuscript).

Species richness and diversity of the small mammal communities showed little change over the decade since treatment. The lower diversity indices on the pre-treatment in 1979 and post-treatment in 1980 can be linked to low vole numbers. Similarly, the lower species diversity on the con-

trol than treatment areas in 1990 is likely due to lower numbers of voles and the absence of chipmunks. This lack of change in diversity is similar to the result reported for small mammal communities in sub-boreal spruce forest (Sullivan et al., unpubl. manuscript).

Thus, this study provides the first truly long-term investigation of the effects of forest herbicide use on demography and diversity of small mammal communities. Despite the lack of suitable replicates, we reject the hypothesis that these attributes would be adversely affected in herbicide-treated habitats at 9 and 11 years after treatment.

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