CILIATES OF SAGEBRUSH-STEPPE SOILS OF SOUTHWESTERN IDAHO: SPECIES RICHNESS AND ITS RELATIONSHIP TO CHANGED SOIL VARIABLES AFTER WILDFIRE EVENTS

by

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The following individuals read and discussed the thesis submitted by student Laura G. Wendell, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

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ABSTRACT

I conducted a pilot study to examine species richness of terrestrial ciliates associated with big sagebrush habitat (*Artemisia tridentata*) in southwestern Idaho, USA. As wildland fires are a naturally occurring disturbance in this area, soil variables associated with these fires (% C, % N, pH, soil texture, and % clay) were measured at three sites, both in burned and unburned areas. These soil characteristics were compared to corresponding ciliate diversity and ratios of *r*-selected colpodeans to K-selected polyhymenophorans (C/P) in non-flooded Petri dish preparations. A total of 85 ciliate taxa were observed across the three sites, including one potentially novel species of *Gastrostyla*. Eleven of the 85 species (13%), seven of which were Colpodeans, were found in all plots. Of the 85 species recorded, 27 occurred in only one plot, and 15 were present only in two. Therefore, 49.4% of species observed within the seven samples were considered rare. C/P ratios and observed species richness were negatively correlated between unburned and burned plot conditions, except for the paired samples from one site. No statistically significant associations between richness and soil variables were found. A significant negative relationship was found between C/P ratios and % clay, with unburned plots experiencing a lower C/P ratio and higher percent clay. This study shows promise for identifying and understanding the role and relationship of terrestrial ciliates both within and to their environment.

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LIST OF ABBREVIATIONS

BLM	Bureau of Land Management
BSU	Boise State University
ICOP	International Congress on Protistology
PCR	Polymerase chain reaction
SSSI	Sagebrush steppe soils of southwestern Idaho
USGS	United States Geological Survey

INTRODUCTION

Ciliates are ubiquitous, single-celled eukaryotes belonging to the kingdom Protista, and are characterized by cilia-bearing basal bodies (at some point in their life cycle), nuclear dimorphism, and sexual reproduction (conjugation). Despite their global distribution in nearly every type of habitat, this large group remains under-studied especially in North America (Foissner 1999a; Corliss 2002). A relatively small number of ciliates, including *Paramecium, Tetrahymena,* and *Euplotes*, are well-known to science and are used extensively as model organisms. However, most recognized species lack characterization by modern methods such as silver impregnation and gene sequencing (Cowling 1994). Ciliates play an important role in both terrestrial and aquatic ecosystems, functioning as predatory grazers in the microbial loop. They feed on algae, bacteria, diatoms, flagellates, fungi, other ciliates, and occasionally members of their own species (Zwart and Brussard 1990; Lynn and Corliss 1991; Foissner 1999a; Corliss 2002). In water-limited environments, they survive desiccation by forming resting cysts (Gutiérrez et al. 2003; Foissner 2011). Encystment is a form of cryptobiosis during which cells extrude unnecessary components and develop a protective, multilayered cyst wall (Corliss and Esser 1974; Fenchel 1987; Foissner 1987, 1993; Lynn and Corliss 1991; Cowling 1994; Gutiérrez et al. 2003).

Difficulties in characterizing ciliates found in terrestrial biomes can be attributed in part to the overwhelming prevalence of encysted ciliates relative to active ciliates at

any given point in time, especially in drier soils (Foissner 1999a). However, increased recognition of the importance of ciliates in soil nutrient cycling is driving researchers to review and improve their methods of studying ciliates in native habitats. For example, field studies are needed to elucidate the ecological role of ciliates in specific habitats, as well as uncover the causes and development or evolution of morphospecies (Corliss 2002). While a few ciliates are found in almost all soils where studies have been carried out, some researchers postulate that there may also be rare species exclusive to specific regions (the cosmopolitan versus "moderate endemicity" models; Foissner 1999b). These rare ciliates can include "flagship" species that are so unusual in appearance it is presumed they are truly endemic to the location in which they are discovered (Foissner 2005). Their unique features are thought to make it impossible to miss them if they were present in other studied habitats (Foissner 1999b).

Sagebrush-steppe is one type of habitat where ciliates have never been studied. This arid ecosystem spans much of the Great Basin, including southwest Idaho, and is characterized by sagebrush and perennial bunchgrasses. Sagebrush-steppe habitat is rapidly disappearing due to overgrazing of understory by livestock, encroachment by invasive plant species such as *Bromus tectorum* (cheatgrass), and the increasing frequency, size, and intensity of wildfires, the majority of which are anthropogenic (Hilty et al. 2004; Jensen and McPherson 2008a). Although wildfires are a natural occurrence in sagebrush-steppe, in the last several decades they have become larger, more frequent, and less manageable (Jensen and McPherson 2008b), and are a direct cause of the decline of big sagebrush, *Artemisia tridentata* (Christensen 1985). The decline of this native species allows an unprecedented efficacious growth rate for cheatgrass and other invasive

annuals, which makes the recolonization or reestablishment of sagebrush challenging. Thus, the cycle continues, causing the degeneration of this once-pristine habitat (Whisenant 1990).

Wildfires vary widely in intensity and severity, which directly influences changes in the soil system (Certini 2005). Fires can create patchiness in vegetation, alter local physical and chemical soil parameters, and change resource availability (Wright and Bailey 1982; White and Pickett 1985). Responses by sagebrush-steppe to burning are influenced by pre-fire fuel moisture, heat transfer through the soil profile, changes in water retention based on alterations in soil characteristics (i.e., compaction, porosity, structure, and texture), nutrient loss or restructure based on volatility during burning and postfire pH changes, and related responses from animal populations such as livestock and small mammals (Wright and Bailey 1982; Christensen 1985; Certini 2005). Some of these variables are known in turn to affect the distribution of protozoa in the soil, as well as vegetation and vegetation cover (Foissner 1987; Cowling 1994; Bowers et al. 1997).

There is a dearth of knowledge regarding the contribution of protist communities to ecosystem function (Cowling 1994; Santoferrara and Alder 2012). There are even fewer studies concerning the response of terrestrial protists to changes in habitat, such as altered soil conditions following fire, and have mostly been limited to the testate amoebae (Wanner and Xylander 2003; Wanner 2012). The fire cycle is dynamic in sagebrush-steppe and can leave a lasting impact based on the severity of the burn (Certini 2005; Brennan et al. 2009). This elicits an ecosystem response (Keeley 2009), that allows us an opportunity to study ciliate diversity in association with this phenomenon.

Species inventories based on detailed morphologic studies are an essential first step in defining the ecological role of ciliates in sagebrush-steppe soils (Foissner 1999a; Boenigk et al. 2012; Lara and Acosta-Mercado 2012; Stoeck et al. 2014). Therefore, in an effort to create baseline information about ciliate populations in sagebrush-steppe soils of southwestern Idaho, my thesis aims to morphologically characterize ciliates that occupy these soils, as well as address Foissner's model of moderate endemicity of ciliate distribution (Foissner 1999b). I also aim to examine associations between the ciliate communities and soils putatively modified by past wildfire events, to gain insights into which physical or chemical environmental factors may be driving any differences in diversity that I observe.

METHODS

Study Sites

My research was conducted from May 2013 to August 2014. I selected three study locations on BLM land in southwestern Idaho that had experienced unplanned wildfires (Fig. 1). Based on BLM records obtained from the Boise Interagency Dispatch Center, I selected sites that had burned one or two fire seasons before the study, but not in the eight years prior. The fires covered 100-199 acres and were ignited by humans. All three sites were in undeveloped habitat that contained stands of big sagebrush *(Artemisia tridentata).* The elevation of the sites fell between 2501-3500 ft. Details of the fire characteristics at each site are provided in Table 1.

Site A was located in western Elmore County (43° 22′ 34.623″, -115° 57′ 7.863″). The topography was relatively flat with the occasional slope. Vegetation was mostly short grass, with sagebrush stands in small patches occurring sparsely throughout. There was visible new growth within the burned plot, and undisturbed patches existed possibly due to fire spotting or area recovery. Sagebrush stands where burns were evident had blackened or charred stems, but rarely the absence of brush or undergrowth.

Fuel at site A had consisted mostly of annual grasses and forbs. Base Line Road runs along the northeastern perimeter of the burned plot, separating it from the control plot. A small section of the area is near a few private homes, and there are some electrical transmission lines and one tower within the burned perimeter. This fire had been

controlled with a fire line around the perimeter without the use of fire retardant (BLM, unpub. data). Based on this, and the appearance of the site at sampling, depth of original burn was most likely “light” (Neary et al. 2008). These details are supported by BLM documentation, which states that fires occurring in this general area are typically low intensity (Bureau of Reclamation 2008).

Site B was located in southwestern Gem County (43° 50′ 29.019″, -116° 37′ 4.799″). The topography was gently rolling and vegetation was mostly tall grass (BLM, unpub. data). Sagebrush stands occurred in patches intermittently throughout the area, though these were noticeably absent towards the western side of the site. Undisturbed patches existed within the burned plot, presumably due to fire spotting or area recovery. Stands where burns were evident had blackened or charred stems and branches, occasionally with absence of brush or undergrowth. The soil beneath was still blackened through the top 1-2 centimeters.

Fuel consisted mostly of annual grasses and forbs. Little Freeze Out Road runs along the eastern perimeter of the burned plot, separating it from the control plot. A short length of chain link fence borders the northeast perimeter, near the fire point of origin. This fire had been controlled with a fire line around the perimeter; no fire retardant had been used (BLM, unpub. data). Based on this, and the appearance of the site at sampling, depth of original burn was most likely “light” to “moderate” (Neary et al. 2008). This is supported by BLM documentation, which states that fires occurring in this general area are typically low intensity (Bureau of Reclamation 2008).

Site C was located in western Ada County (43° 19′ 55.897″, -116° 24′ 38.624″). The topography was relatively flat with a slight slope along the northeastern fire

perimeter. Vegetation was mostly sagebrush in stands, except along the northern side where it was mainly grasses. Fuel consisted mostly of sagebrush and annual grasses (BLM, unpub. data). Sagebrush plants had blackened or charred stems, mostly with absence of brush or undergrowth. Occasionally the aboveground shrub material was lacking, except for a short stump and mound. Throughout the entire burned plot, new growth was limited or absent. The soil was still black in the top few centimeters of sampling.

South Swan Falls Road runs along the western perimeter of the burned plot, but no control replicates were collected from the other side of it. No structures were present on the site. A fire line around the perimeter was used to control the fire; fire retardant had been used. Based on this, and the appearance of the site at sampling, the depth of original burn was most likely “moderate” to “deep” (Neary et al. 2008). This is supported by BLM documentation, which states that fires occurring in this general area are typically mixed intensity (Bureau of Reclamation 2008). The area was considered critical habitat for endangered species.

Soil Sampling and Microcosm Establishment

At each of the three study sites, I selected 10 locations at which to sample for soil conditions and ciliates, five sampling locations within burned areas each with a nearby sampling location outside of the burned area (Figs. 2-4). These were maintained within approximately 100 m of each other, to create a “pair,” though occasionally this was not possible due to limitations with the distribution of sagebrush stands. To minimize the possible variation caused by vegetative characteristics at the site, a sampling location was deemed suitable only if it contained at least two sagebrush plants (or their burned

remains) with 1.5-6 meters of soil between shrubs (interspace). I used a hand-held GPS to record the GPS coordinates of all sampling locations.

At each sampling location, I took two soil samples: a shrub-mound sample (i.e., soil at the base of a sagebrush) and an interspace sample. Each sample consisted of 0.6 m diameter section of soil (including grass, roots, moss, and other organic material) taken from the surface and litter layers to a depth of 10 cm using a trowel and/or shovel. Each sample was stored in a gallon Ziploc bag and labeled by site, sample number (1-5), burned or control plot (*b* or *u*), and mound or interspace (*s* or *i*). Samples were weighed onsite using a commercial hand-held fish scale to ensure that I collected at least 0.5 kg of soil and litter, and were then returned to Boise State University where they were stored in a limited access laboratory, open to the air, for at least 4 weeks (maximum = 16 weeks). All sampling was conducted over a two-week period in May 2013.

After at least 4 weeks of storage in the laboratory, soil microcosms were established following the non-flooded petri dish protocol, following Foissner (1991) with slight modification. I first homogenized the dried soil in each sample manually to break down large soil-root masses and clumps. I then combined equal amounts of soil from the interspace and shrub-mound samples collected at each sampling location. These samples included leaf litter and other organic material. Mixed shrubmound and interspace soil (165-183 g dry weight), comprised of approximately 70% soil and 30% litter, was placed in large glass Petri dishes (15 x 3 cm) to a depth of 1.5 cm. Remaining mixed soil was stored separately in paper bags for all following soil analyses, and any subsequent duplicate dishes. The soil in the dishes was then moistened with distilled water, covered,

and left for 30 days at room temperature to allow the ciliate community to develop. Soil was rewetted as required to maintain soil saturation and produce a percolate.

Soil Analysis

The 10 soil samples (5 burned, 5 unburned) taken at each site were sieved to produce soil particles no more than 2 mm in diameter. Samples within burned and unburned conditions from each site were pooled to create a total of six samples weighing 2 g each for analysis of total carbon (C) and nitrogen (N) content. These samples were pulverized to a uniform powder of approximately 1 um using a SPEX ball mill and analyzed on a Thermo Scientific Flash EA 1112 NC soil analyzer. Internal standards consisted of Thermo Scientific NC soil reference material and aspartic acid.

Measurements of pH and analysis of soil texture were made on the dry soil remaining after the establishment of microcosms. Method 4C1a2a1a-b1 from the Soil Survey Laboratory Methods Manual (Soil Survey Staff 2004) was adapted to determine pH by 1:1 soil weight: volume H_2O without the use of an automatic titrator. Soil weighing 20 mg was measured into a glass beaker and 20 ml of nanopure water was added. The mixture was stirred on a stir-plate for 30 s in 15-minute intervals over the course of an hour. A pH meter was immersed in the liquid fraction above the soil after one minute of settling.

Determination of soil particle size was made using the “rapid method”: a simplified texture analysis protocol that is strongly correlated to more traditional pipette techniques (Kettler, Doran, and Gilbert 2001). Approximately 15 g of <2 mm soil was suspended and shaken in 3% aqueous hexametaphosphate for 2 hours at 120 reciprocations per minute. The sand fraction was separated out, oven-dried, and then

weighed on a bench scale and recorded within 10 milligrams. The silt and clay fractions remaining were allowed to settle between 90 min and 2.5 hours. The silt fraction was separated out and treated as per the sand fraction. The clay fraction was calculated as the percent sample remaining.

Ciliate Diversity: Microcosm Sampling and Slide Preparation

Apart from a few minor exceptions described at the end of this section, ciliate diversity was determined for only one pair of the five pairs of soil samples taken at each site, i.e., one sample from a burned area along with its unburned counterpart. As ciliate diversity in microcosms is reported to be highest within the first few days and weeks of establishment (Foissner 1991; Schwarz and Frenzel 2003), on days 1, 2, 7, 14, 21, and 30 I collected enough sample from each of the six dishes to make eight slides. Thus, for each of the study sites (A, B, C) and treatments within sites (burned, unburned), I made a total 48 slides over the 30-day period.

Identification of ciliates was based on morphostatic (i.e., non-dividing) forms primarily through the use of silver-impregnation (protargol) of fixed material, using the taxonomic keys of Foissner (1993), Foissner et al. (2002), Foissner and Xu (2007), and Berger (1999, 2006, 2008). In cases where species identification was difficult based on the fixed samples, I returned to the original soil sample and created an additional 'duplicate' microcosm. This provided additional material from a given time-point (i.e., collection day 1, 2, 7 etc. when they were initially found) for more detailed study of the specimens in question.

Two different protargol methods were used, both with modifications to published protocols. Method "A" (Foissner 1991; Dieckmann 1995), which I used on material from

the original petri dishes, created permanent fixed slides that could be used indefinitely. Method “B” (Wilbert 1975; Foissner 1991, 2014; Song, pers. comm.), a more intensive procedure, was used on material from duplicate petri dishes only. This method produced slides that were suitable for use only for a short period of time. I used this approach in two instances to verify identification of specimens that were difficult to identify using Method A.

In preparation for protargol protocol “A” (a modified protocol based on Foissner 1991), approximately 5 ml of soil percolate was collected by sweeping the pipette along the edge of a tilted dish while rotating continuously, and finally aspirating gently straight from the soil surface. I used a “high flow-through” protocol for staining the slides. Initially, protargol was synthesized using methods from Pan et al. (2013). Slides were impregnated with this protargol using a modified protocol combining elements of the Vd’ačný and Foissner method (2012) and the Dieckmann method (1995), as described in Appendix A.

In the two cases where protargol method “B” was required, I used Wilbert’s (1975) method along with minor modifications suggested by Foissner (1991, 2014) and Song (pers. comm.). Ciliate specimens from duplicate dishes were removed using a micropipette under a stereoscope and fixed in an embryo dish in an equal amount of Bouin’s fluid. Once several specimens were collected in the same vessel, they were bleached using a 0.01% solution of commercial bleach. Cells were impregnated with 1% protargol on a 60°C hotplate and developed with “ordinary” developer (Vd’ačný and Foissner 2012). Permanent preparations were treated with thiosulphate, washed and dehydrated as described above.

After staining, all slides were examined under an Olympus BX53 microscope using light, phase, or differential interference contrast under 60X objective magnification, or under 100X power using oil immersion. Microphotographs were taken with a Canon EOS digital camera. Measurements were made using a calibrated optical micrometer. I prepared a list of all species I could identify from my samples, and specimens that could not be identified to species were classified to the next most descriptive taxon.

In addition to the 288 slides described above (8 X 6 X 6), I made another set of 48 slides from a second burned location at site C, as well as 24 slides from several randomly selected locations at the three study sites (total slides = 360) to provide additional information about heterogeneity of ciliate communities within sites. These slides were prepared using protargol Method A.

Ciliate Diversity: Analyses

I constructed a species accumulation curve to estimate the total number of ciliates present at the three sites based on the 360 slides I examined. I estimated expected species richness using the nonparametric test statistic Chao2 as calculated in EstimateS (Colwell 2013). Chao2 is used to detect the lower bound of the asymptote of a species richness curve for incidence-based data (Chao 1987), which, given the sample size in my study, provides a better fit than the incidence-based coverage estimator (ICE) as Chao2 has a small sample-size adjustment factor in EstimateS (Colwell et al. 2012; Colwell 2013). Using EstimateS (Colwell et al. 2012), I made rarefaction curves to determine how many samples might be required to reach the asymptote of species richness if it had not been reached. These curves were based on soil samples taken at the seven locations (i.e., two pairs at sites A, B, and C, plus the additional location at site C). I used a rank-abundance

curve (Hughes et al. 2001) to visualize frequency of species in the ciliate community. Species that occurred only once in sagebrush-steppe soils of southwestern Idaho (SASI) were used to detect which plots were contributing unique ciliates to reflect a possible difference in rate of richness accumulation between sites.

Bartošová and Tirjaková (2008) categorized ciliates species by systematic class (Lynn and Corliss 1991) and compared them to Foissner's (1998) accounting of global ciliate diversity. I repeated this approach to determine the extent to which ciliates in sagebrush-associated soils in southwestern Idaho are represented globally. Additionally, I determined the C/P ratio—i.e., the number of colpodean ciliates (r-selected Colpodeans plus the Spirotrichean genera *Paraholosticha*) relative to polyhymenophorans (K-selected Heterotrichea and all other Spirotricheans) in both burned and unburned samples. This ratio has been used by others (Luftenegger et al. 1985; Bamforth 1995; Bartošová and Tirjaková 2008) to estimate the stress of an environment. "Extreme" soils, such as those found in arid and semiarid deserts, often have a greater C/P ratio than soils that are not limited by water or abundance of food (Luftenegger et al. 1985; Bamforth 1995; Foissner et al. 2002).

I used correlation analyses to examine the relationships between soil characteristics of burned and unburned soils as they relate to ciliate species richness and site-specific C/P ratios. Correlations were evaluated using Pearson's product-moment correlation, and tested with student's t. Nonmetric multidimensional scaling (NMDS) and hierarchical clustering using unweighted pair-group with arithmetic mean (UPGMA) analyses were carried out using the vegan package (Oksanen et al. 2011) in R (R Development Core Team 2014) to measure differences in richness of sites (beta diversity)

based on Bray-Curtis (Bray and Curtis 1957) and Jaccard (Jaccard 1912) distance matrices, respectively.

RESULTS

Overall Species and Community Composition

I detected a total of 85 ciliate species across the three study sites (Table 2), of which some of the diversity is shown in Appendix A. Permanent protargol slides (Method "A") yielded 83 of the 85 species; the remaining two species were isolated and identified using protargol "B" from duplicate dishes. Eleven of the 85 species, or 13%, were found in all plots; these were *Colpoda inflata, C. maupasi, C. steinii, Cyrtolophosis mucicola, Gonostomum affine, Grossglockneria acuta, Homologastra setosa, Leptopharynx (costatus) costatus, Platyophrya vorax, Protocyclidium muscicola,* and *Pseudoplatyophrya nana.* All of these were found across all seven samples, except *P. muscicola* and *H. setosa*, which were found only in six. Seven of the nine found in all samples were Colpodeans.

Both the species accumulation curve (Fig. 5) and rarefaction curve (Fig. 6) indicated that although a majority of expected ciliate species were discovered through sampling, the asymptote of species discovery had not been reached. The rarefaction analysis provided an estimate of approximately 105 species expected at my sites; the Chao2 richness estimator indicated 104 species expected by the 22nd sample. Thus, from my seven samples, I discovered approximately 82.5% of the expected species richness of the ciliate community. The rank-abundance curve (Fig. 7a) does not show a distinct extended tail for species occurring only in a single replicate; samples contributing

uniques can be seen in Fig. 7b. Out of 85 total species, 27 occurred only once, and 15 were present only twice.

Table 3 shows the taxonomic assignments of all ciliates found in my study, compared to those identified by Bartošová and Tirjaková (2008) in their study of decaying wood moss in Slovakia, and Foissner's (1998) global census. Colpodea, Litostomatea, and Spirotrichea represent the top three classes of ciliates found in all three studies. These three groups captured 75.7% and 80.41% of total diversity found in Foissner (1998) and Bartošová and Tirjaková (2008), respectively, and 88.1% of the diversity found in my study. The four other classes found in all three studies (Phyllopharyngea, Ollygohymenophorea, Nassophorea, and Heterotrichea) represent only a fraction of the overall diversity found in each of the studies. One class, Prostomatea, was not found by Bartošová and Tirjaková (2008).

Site-Specific and Treatment-Specific Species Richness

Rarefaction curves of species richness by site are depicted in Fig. 8a. Sites A and B had two samples each, whereas site C had three (two replicates from burned, one from unburned). At sites A and B, the number of species found was close to the predicted asymptote for total species. Chao2 estimates for site A place total species richness at 66 species by the fourth sample, which indicates that 94% of expected species were observed with only two samples. At site B, this value was 65 species by the fifth sample, 80% of which I saw by the second sample. Site C is predicted by the rarefaction analysis to have higher species richness than the other sites: 86 species by the 24^{th} sample. Only 60% of the expected number of species was encountered in my three samples at this site.

Comparing samples from burned and unburned areas (Fig. 8b), the Chao2 estimator is calculated at 86 species by the 13th sample for burned soil, 76% of which was observed by the fourth sample. In the unburned soil, 81 species are expected by the 6th sample, 89% of which were observed by the third sample. Thus, it appears that unburned soils have more stable populations of ciliates, which requires fewer samples to capture overall diversity.

In all but one instance, C/P ratios were lower in unburned samples (Au=1.33, Bu=1.71, Cu=1.29) than burned samples (Ab=3, Bb=3.5, Cb2=2.57) (Fig. 9). The paired, unburned sampling location at site C (Cb) was an anomaly to this pattern; the C/P ratio in this sample was 1.11, lower than all other C/P ratios recorded.

Beta Diversity

Site C shows evidence of heterogeneity in species composition based on the differences I found between the two samples from the burned areas. Sample Cb had 24 species and Cb2 had 37, for a total of 48 species combined (only 14 of which, or 29%, are shared between the two). The single unburned sample at site C appears low in observed species richness, which indicates that the high value of expected richness from site C may originate from species collected at the burned plot. These observations indicate diversity differences in site C compared to sites A and B. Nonmetric multidimensional scaling (NMDS) analysis shows that five of the seven samples clustered together in terms of species diversity, whereas the paired samples at site C (Cu and Cb) do not (Fig. 10a). However, caution is warranted because stress values were near zero, indicating that there may be insufficient sample size to detect differences among samples. The axes for the NMDS plot have been ordinated such that NMDS1

represents the greatest differences in samples, and NMDS2 the remaining variation (analysis was reduced to *k*=2 dimensions only).

A similar conclusion can be drawn from the results of the clustering diagram using unweighted pair-group with arithmetic mean (UPGMA) analysis (Fig. 10b). Samples Cu and Cb cluster together, well apart from the cluster that includes the remaining five sample locations. Height, the Y-axis in Fig. 10b, indicates the amount of dissimilarity between groupings.

Soil Analyses and Comparison to Ciliate Community Composition

Soil characteristics are summarized in Table 4, by plot (including all 5 sampling locations) and sample used for ciliate analysis. Sites A and B consisted of sandy loam, whereas site C consisted of loam. Percent clay was relatively consistent between burned and unburned samples at sites B and C. At site A, % clay was substantially lower in the burned sample compared to the unburned sample. Soil pH values were circumneutral (6.49-7.31) across sites, with sites A and B being only slightly acidic, and site C slightly alkaline. There was no trend in terms of pH values (i.e., higher or lower) between burned and unburned samples within sites. Within sites, % carbon was higher in burned areas than in unburned areas (note: values within burned areas and within unburned areas were each based on the five pooled samples taken at each site); however, the values differed across sites. Percent nitrogen, again based on pooled samples within sites, was higher in burned areas than unburned areas at sites B and C, but did not differ between burned and unburned areas at site A.

Results differed slightly for soil measurements specific to the sampling locations used for ciliate analyses. The locations used from Ab, Bb, and Cu had soil textures of fine

sand, loamy fine sand, and sandy loam, respectively. Percent clay varied by more than one significant difference for Ab, Bb, Cu, and Cb2. Sampling locations Bu and Bb had pH values also outside the margin of one significant difference.

No statistically significant relationship was found between species richness—whether grouped by sample, by plot or by site—to any of the soil characteristics I evaluated (correlation analysis, all $p>0.05$). I found a significant negative correlation between C/P and % clay in the seven samples (Fig. 11; $r = -0.801$, $t = -3.04$, $df = 5$, $p = 0.029$). No other significant correlations between soil characteristics and C/P ratio were found. pH values show a possible positive, but not significant ($p > 0.05$), relationship with %C and %N values for the unburned samples (Fig. 12).

DISCUSSION

Species Richness and Diversity

In the present study, 85 terrestrial ciliate species were recovered from sagebrush-steppe soils in southwestern Idaho. Nearly all represent first reports of these taxa from Idaho. Almost half (49.4%) of species observed were found in only 1 or 2 samples; however, this determination may be a reflection of the small sample size (i.e., species observed infrequently within these samples) rather than a distinct measure of rarity (i.e., those species that actually occur infrequently in SSSI). In reality, many truly rare species of ciliates likely went undetected in my study. As is evident from the rank-incidence curve (Fig. 7a), the limited tail from observations of unique species supports the conclusion that the soils in my study were under sampled, as a high frequency of rare ciliates should be observed in soils that have been extensively sampled (Foissner 1998).

All of the ciliates I observed in my study had been previously described, with the possible exception of a new species of *Gastrostyla*. Additionally, the common ciliates found across all (or most) of these replicates are very similar to the most frequently found ciliates in studies done worldwide. Five of the most common ciliates (*Colpoda inflata, C. maupasi, C. steinii, G. affine,* and *P. nana*) recovered from Namibian soils (Foissner et al. 2002) were also the most common in my study. These results are not surprising, given that these species are considered generalists (i.e., well-adapted to survive in most environments and not limited by food specificity) (Luftenegger et al. 1985; Bamforth

1995). A similar community of widely distributed ciliates (*C. inflata, C. steinii, C. mucicola, G. affine,* and *L. costatus*) has been reported from soils of flooded anoxic rice fields in Italy (Schwarz and Frenzel 2003).

Although the ciliate community in my study was nearly identical to that found in Namibian soils in terms of the most frequently observed taxa (Foissner et al. 2002), I observed only one potentially novel species (*Gastrostyla* sp.). By contrast, 128 of 365 species (i.e., 35%) of taxa reported in Foissner et al. (2002) were newly described. Despite observation of so-called endemic "flagship species" (Foissner 2005) in other Idaho terrestrial habitats (Bourland 2008), no such species were observed in my study. However, over 70 samples were investigated from Namibia; the rarefaction analysis (Fig. 6) indicates that I have not yet reached the asymptote of species richness, which is where the undiscovered species may exist.

Therefore, given the small sample size in my study, it is not possible to lend support either toward the cosmopolitan theory of global protist distribution (Fenchel et al. 1997; Finlay and Fenchel 1999), which holds that ciliates are far less geographically restricted than their larger counterparts in the animal kingdom, or the moderate endemicity model (Foissner 1999b; Foissner et al. 2007), which promotes the notion that a fraction of ciliates are endemic to the region in which they were discovered. The samples I collected were enough to identify the cosmopolitan species (i.e., those found worldwide), but not the species only sparsely distributed in these sagebrush soils.

From a practical standpoint, given that sampling from each of the microcosms extended 30 days, sample fixation took 2 hours, the staining of 16 slides took up to 3 hours, and examining *each* slide took anywhere from 3 to 16 hours; the sampling effort

that would be needed to capture the majority of rarer species would require a considerably larger workforce and/or extended period of study. Thus, questions requiring knowledge of the entire ciliate community would necessitate implementation of alternate techniques, if feasible at all. Use of molecular techniques would allow identification of the ciliate community beyond the morphospecies concept, where additional diversity may be present (Katz et al. 2011).

Small sample sizes can create difficulty when interpreting observed differences in species richness across samples. Even with all seven samples included, the confidence intervals on the total species richness curve (Fig. 6) are large. Based on my rarefaction analysis, the 85 species I discovered represent the lower bound of a ciliate community that may exceed 120 species. Moreover, heterogeneity in community composition within plots, as seen in Cb and Cb2, make interpretation of the extrapolations based on a single sample per plot (two per site) questionable. The large confidence intervals in the rarefaction curves for site C (Fig. 8) are likely the result of having two (dissimilar) samples, rather than one sample, in burned areas. The smaller confidence intervals for sites A and B may simply reflect the fact that only two samples from each of these sites are included in the analysis. If substantial heterogeneity in the ciliate community exists within those sites, as occurs in site C, then the addition of more samples would increase the confidence intervals of those curves, and most likely the expected richness value. From this, I again conclude that many more samples would be needed to adequately census the ciliate community because the Jaccard and Sorenson indices of dissimilarity in diversity are sensitive to both sample size and the fraction of rare species observed (Wolda 1981; Plotkin and Muller-Landau 2002).

Ciliates from the class Spirotrichea were the most prevalent group in the global study by Foissner (1998) and the DWM study by Bartošová and Tirjaková (2008). As a result, the C/P ratio in those studies was *less* than 1. By contrast, in my study of ciliate communities in arid soils in Idaho, colpodeans were the most common group, resulting in a C/P ratio of >1. Indeed, even at the species level the majority of specimens in my samples belonged to this class. Colpodeans excel at forming cysts, and thus are well suited for life in arid soils. In fact, members of the genus *Colpoda* divide only in an encysted state (Foissner 1993; Lynn 2008), which indicates a high level of specialization to life in stressed environments.

Not surprisingly, I found that ciliates from burned samples were nearly 3:1 colpodeans to polyhymenophorans, approximately twice what was observed in unburned soils. This suggests that the burned plots, albeit 1-2 years following fire disturbance, are more stressed environments for ciliates than the surrounding unburned plots. However, ratios are known to vary in arid soils, measuring anywhere from 0.35 to as high as 9 (Robinson et al. 2002; Bamforth 2004, 2008), although usually greater than 1 (Luftenegger et al. 1985; Foissner et al. 2002). Due to that variance, as is a common theme from this study, more support from additional samples would be needed to verify interpretations of stress.

Relationships to Soil Environment

The lack of significant relationships between most of the soil characteristics I measured and C/P ratios were not surprising given the deficiency in sample size in this study. However, I did find a significant negative relationship between condition-dependent (burned or unburned) C/P ratios and percent clay across samples. Clay tends to

increase the soil's water holding capacity, and it has effects on cation exchange capacity, soil thermodynamics, aggregate structure, and adsorptive properties (Mukherjee 2013). Some of these variables may impact pore space, which in turn impacts protist community structure (Finlay and Fenchel 2001). However, without analyzing more physical or chemical properties related to fire or fire recovery, such as soil structure, porosity itself, or organic matter (Certini 2005; Neary et al. 2008; Bento-Gonçalves et al. 2012; Badía-Villas et al. 2014), it is difficult to determine what may be driving the observed correlation, or even if it is related directly to the fire disturbance (Adl and Gupta 2006). For example, livestock grazing histories for these sites are not known, which along with fire may be influencing some of the physiochemical differences observed between sites and samples within sites (Jensen and McPherson 2008a).

Additionally, it's possible that no trends in soil characteristics were seen due to the proximity to the fireline from which we were sampling "unburned" samples. If a homogenization of topsoil occurred at those nearby unburned and burned areas due to the effects of wind, etc, then it would be reasonable to assume that physical and chemical characteristics would be similar, and the ciliate community may reflect that. Unfortunately, no measure of the scale by which terrestrial ciliate diversity changes locally is known. If a difference exists between species richness and environmental parameters at different locations, the distance that appropriate sampling would need to span in order to detect this remains to be discovered.

Methodological Limitations

The initial goal was to sample 5 paired locations at sites A, B, and C to obtain a total of 30 samples (15 burned and 15 unburned) for analysis, both in ciliate inventory

and soil characteristics. As has been previously mentioned, the amount of time required to complete a single sample from each condition within each site for indexing ciliate species prohibited the analysis of remaining collected samples. At the one site where a second burned sample (Cb2) was actually inspected, the species inventory was vastly different from the first studied sample (Cb), which can't be explained by differences in soil characteristics (Table 4b). This is reflected in Fig. 8a, where the rarefaction curve for site C predicts a higher number of species than for the other two sites. Excluding that second burned sample from site C may very well have led to a curve similar to those seen in the other sites, which means conclusions drawn from that analysis are of limited value. The appearance of reaching the asymptote in these curves is more representative of the detection rate within the samples I examined, rather than the plots or sites as a whole.

From the seven samples together, 82.5% of ciliate species (based on the Chao2 estimate) were discovered in sagebrush soils in southwestern Idaho. This does provide a good starting point for future studies involving generalists in these soils, or even for those studies directed at determining possible bioindicators. Some of the non-generalist species catalogued could be easy to detect in field studies monitoring ciliate response variables. Studies looking for more power in community analysis, especially over multiple sites, would receive validation from the results seen here to employ molecular tools for environmental samples, such as pyrosequencing, which have begun to find their way into protistological research (Jousset et al. 2010; Lie et al. 2014). These provide mass amounts of DNA sequences that can be used to determine taxa present in the soil where the samples were collected. However, limitations to our understanding of intra and inter-specific molecular heterogeneity in ciliates (Katz et al. 2011; Gong et al. 2013) are the

next hurdle to overcome when adding these tools to future studies. Having a compilation of at least 80% of expected ciliates in SASI soils, as accomplished by my study, will aid in corroborating the vast amount of data those next generation techniques (such as pyrosequencing) produce.

In my study, soils collected from beneath sagebrush plants were pooled with interspace soils. Interspace soils may support fewer species than shrubmound soils (Robinson et al. 2002) and those associated with the shrub rhizosphere (Zwart et al. 1994). While not a focus of this study, any differences in shrubmound or interspace ciliate communities would have gone undetected. On the other hand, pooling soil samples still allows for a cumulative tally of ciliate species, and given the paucity of information about ciliate communities in general, the knowledge gained in my study provides a starting point for more detailed analyses of the effects of soil conditions on ciliate community structure. Such studies are warranted given that there is mounting evidence in the literature for patterned heterogeneity in microbial eukaryotic communities in soil (Acosta-Mercado and Lynn 2002; Green et al. 2004).

Some of the cluster distances (Fig. 10b) with regard to ciliate species composition at the study sites may be attributed to vegetation differences at those sites (short grass, tall grass, or shrub). For example, Zwart at el. (1994) report protist-plant specificity in their study of soil nutrient dynamics, though with high variation. Gel'tser (1992) argued that protist species composition was specific to different plants and their roots. Acosta-Mercado and Lynn (2004) recorded the same observations from studies examining the ciliate community associated with rhizosphere environments from tropical plants. Though I collected the samples from the soil surrounding sagebrush, it is possible the ciliate

community is impacted by neighboring vegetation. In order to tease out influences the neighboring vegetation might have, I would recommend elimination or restriction of heterogeneity in soil conditions in future collections.

Further, my method cannot detect a difference between active ciliates or encysted ciliates. Studies have indicated that cysts can be reactivated from soil after more than three decades (Goodey 1915; Moon-van der Staay et al. 2006). Therefore, certain species may be able to withstand unfavorable conditions in the soil for a prolonged period of time while maintaining cryptobiosis. By examining the soil after *all* ciliates have encysted, as was required by my methods, the ciliates that then excyst in the lab may not reflect changes in ciliate species that become active in soil after disturbance events (Adl and Gupta 2006). Additionally, not all of those who successfully encyst will survive; it has been calculated that sometimes as little as 25% of those who began the encystment process will effectively excyst and survive to repopulate (Ricci et al. 1985), which highlights yet another pitfall in using this method to examine an entire soil ciliate community.

The methodology employed in my study has limitations when it comes to characterizing species richness of entire ciliate communities (Foissner et al. 2002). For example, some species may not be collected in high enough numbers to get around the inefficient excystment of an individual, others may not survive the collection process, and some may never encounter conditions in the dish that promote their excystment (Foissner 1997, 1999b; Schwarz and Frenzel 2003; Foissner et al. 2005). Nevertheless, although the microcosm method had its shortcomings, I was able to detect many of the ciliate species described by others as ubiquitous, thereby bolstering that assertion and adding to the

general knowledge of ciliate distribution. Moreover, many of the species documented in my study represent the first time they have been reported in both North America and Idaho. However, a complete understanding of the SASI ciliate community, its response to disturbance events, and its relationship to the environment will require additional techniques including molecular characterization (Bourland et al. 2011, 2014; Bourland et al. 2012).

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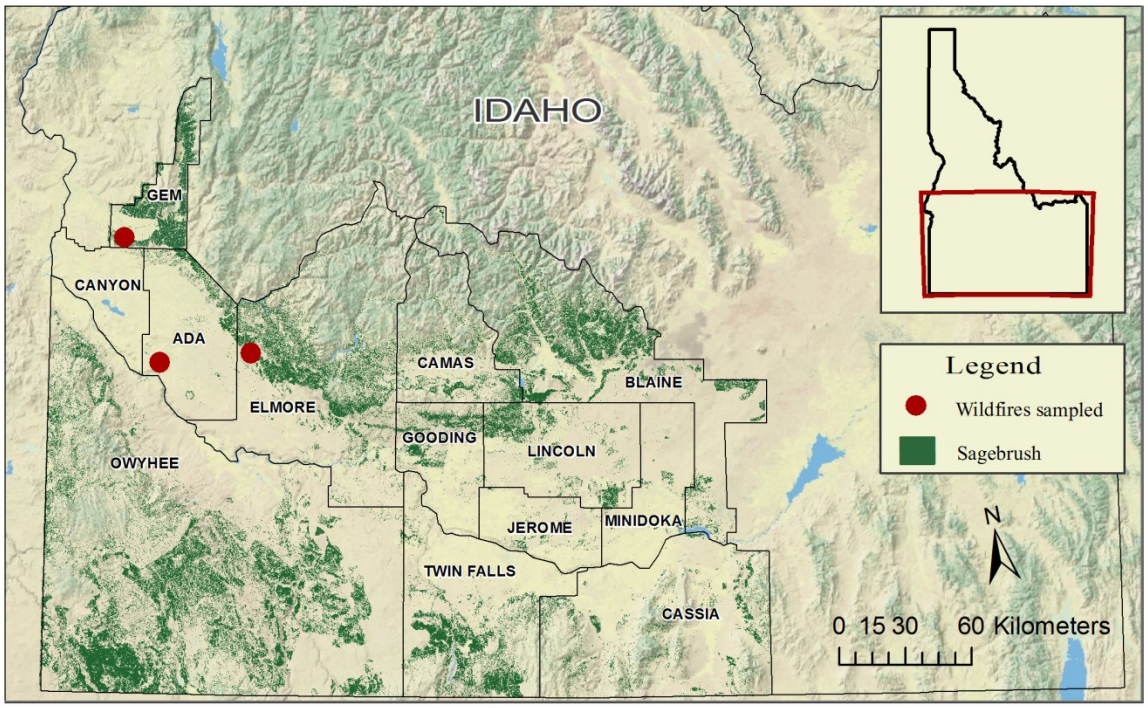

Fig. 1. Counties of southwestern Idaho, USA. Dark green indicates location of sagebrush; red circles are overlaid on wildfire sites from previous fire seasons chosen for this study.

Table 1. Characteristics of fires from burned plots at study sites.

PARAMETERS	FIRES		
	A	**B**	**C**
Burning Index[a,b]	21 (Low)	37 (Moderate)	56 (High)
Est. Flame length at head of fire (ft)[c,d]	2.1	3.7	5.6
Est. Fireline Intensity (BTU/ft/sec)[c,d]	39	94	246
Fuel model[a]	short grass (1 ft)	tall grass (2.5 ft)	brush (2 feet)
Est. Fuel loading (tons/acre)[d]	1 h- 0.74	1 h- 3.01	1 h- 1
	10 h- 0	10 h- 0	10 h- 0.5
	100 h- 0	100 h- 0	100 h- 0
Est. Fuel moisture[b,e]	12%	25%	20%
Est. Fuel bed depth (ft)[b,e]	1	2.5	2
Fire regime[a,f]	low severity	low severity	mixed severity
Typical return interval (years)[f]	0-35	0-35	35-100
Burn start date[a]	7/6/2011	8/5/2012	9/22/2012
Duration (hr,approx)[a]	76	54	53
Weather - Temp (°C)[g]	94	90	71
Relative Humidity[g]	16%	16%	30%
Wind Speed (mph)[g]	4	5	5
Fuel temp[g]	115	NA	NA
Fuel moisture (gm)[g]	6	NA	NA
Total Size (acres)[a]	118	101	209
Location (County)	Elmore	Gem	Ada

[a]=BLM, unpub data
[b]=Bureau of Reclamation 2008
[c]=Deeming et al. 1977
[d]=Albini 1976
[e]=Anderson 1982
[f]= Neary et al. 2008
[g]=RAWS data from WIMS stations (102709, NWS/FAA KBOI)

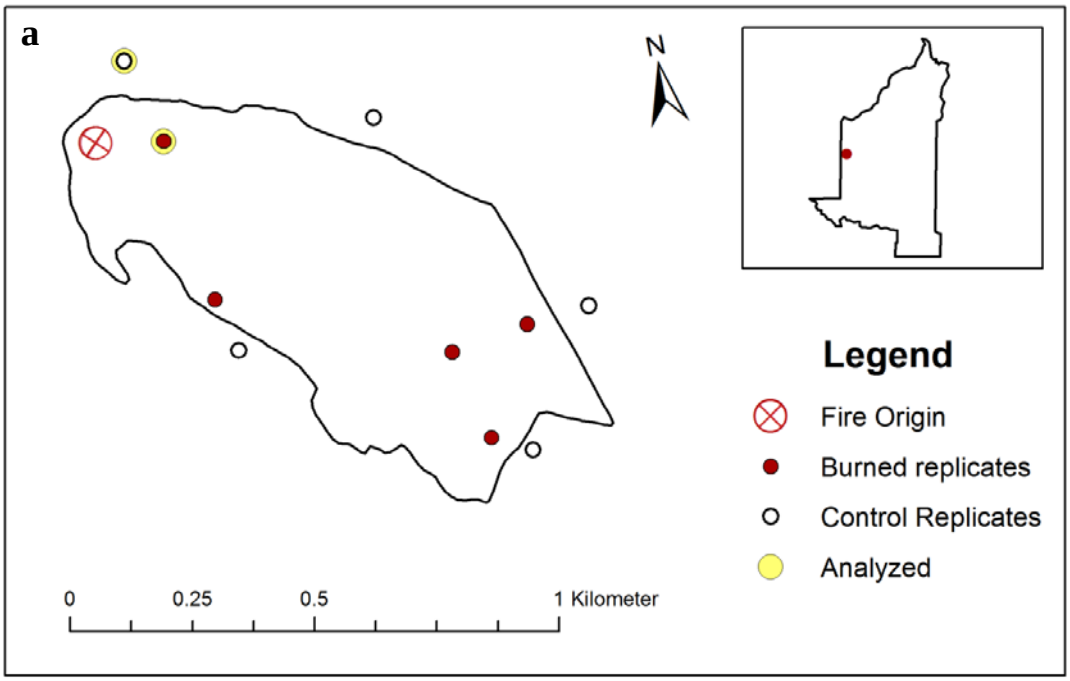

Fig. 2. Study site A: (a) Map of fire margin with location of burned (red circles) and unburned (open circles) sample collection. Samples analyzed for ciliate species richness outlined in yellow. Location of site indicated in inset. (b) Example of burned sampling location, indicating typical condition of shrubs and composition of vegetation.

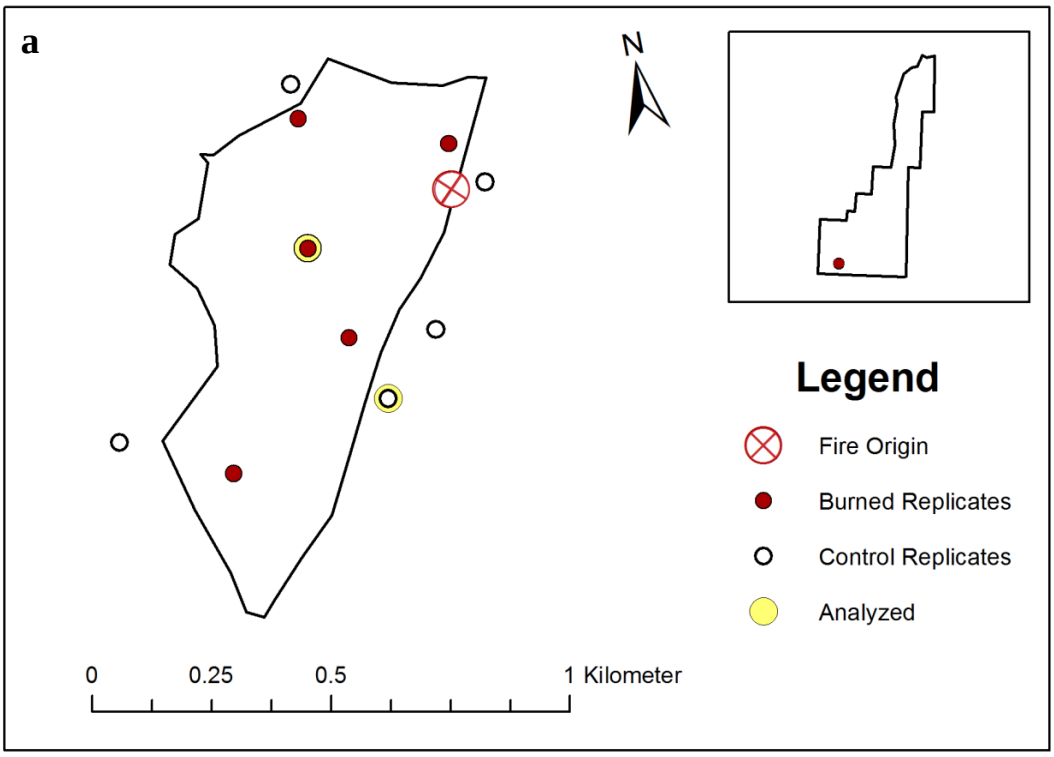

Fig. 3. Study site B: (a) Map of fire margin with location of burned (red circles) and unburned (open circles) sample collection. Samples analyzed for ciliate species richness outlined in yellow. Location of site indicated in inset. (b) Example of burned sampling location, indicating condition of typical shrubs and composition of vegetation.

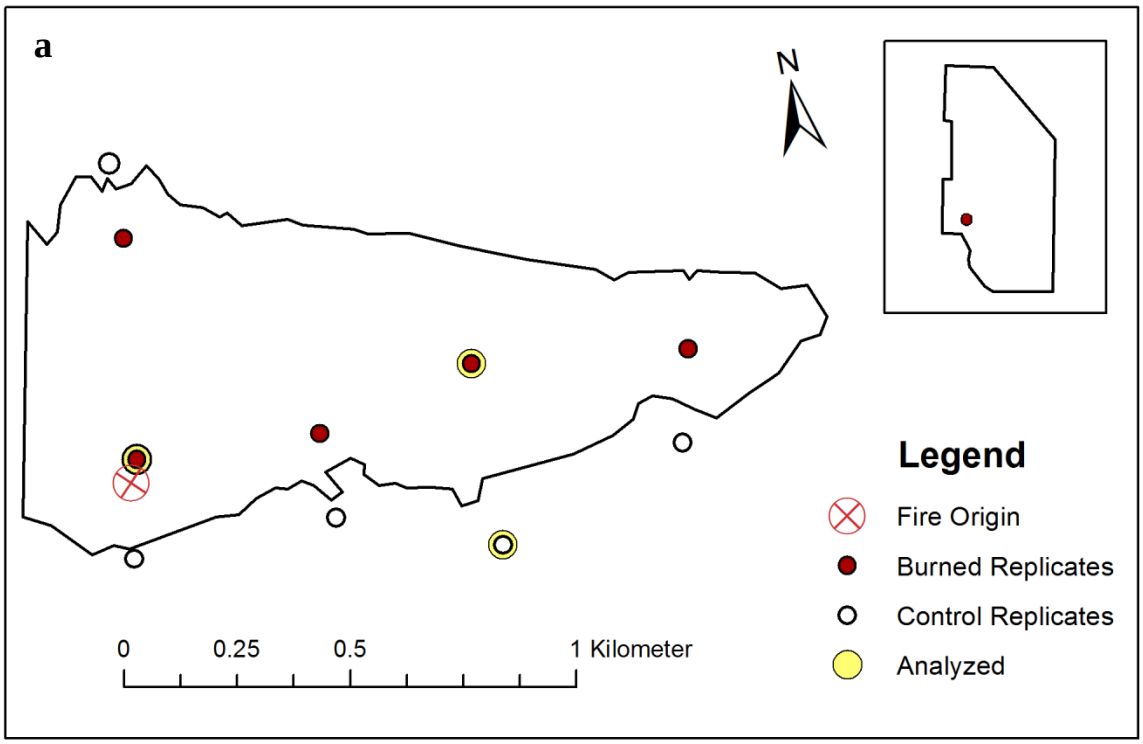

Fig. 4. Study site C: (a) fire margin with location of burned (red circles) and unburned (open circles) sample collection. Samples analyzed for species richness are outlined in yellow. Location of site indicated in inset. (b) Example of burned sampling location, indicating condition of typical shrubs and composition of vegetation.

Table 2. Distribution of species observed across replicates from sagebrush-associated soils of southwestern Idaho. Presence is indicated by (+), absence by (-).

Species	Sample						
	Au	Ab	Bu	Bb	Cu	Cb	Cb2
Anteholosticha heterocirrata (Hemberger 1985) Berger 2008	-	-	+	-	-	-	-
Apospathidium atypicum (Buitkamp & Wilbert 1974) Foissner, Agatha & Berger 2002	-	-	-	-	-	-	+
Arcuospathidium (muscorum) muscorum (Dragesco & Dragesco-Kerneis 1979) Foissner 1984	+	+	-	+	-	-	+
Arcuospathidium (namibiense) tristicha Foissner, Agatha & Berger 2002	-	-	-	-	-	-	+
Avestina ludwigi Aescht & Foissner 1990	+	+	+	+	+	-	-
Balantidiodes muscorum Kahl 1932	+	+	+	-	-	-	-
Birojimia muscorum (Kahl 1932) Berger & Foissner 1989	-	-	+	-	-	-	-
Bistichella procera (Berger & Foissner 1987) Berger 2008	+	+	+	-	-	-	-
Blepharisma hyalinum Perty 1849	+	-	+	+	+	-	-
Caudiholosticha tetracirrata (Buitkamp & Wilbert 1974) Berger 2003	+	-	-	-	-	-	-
Circinella sp. Foissner 1982	+	-	-	-	-	+	-
Circinella filiformis (Foissner 1982) Foissner 1994	-	-	-	-	+	+	-
Colpoda aspera Kahl 1926	+	+	+	+	-	-	+
Colpoda cucullus (Müller 1773) Gmelin 1790	+	+	+	+	-	-	+
Colpoda ecaudata (Liebmann 1936) Foissner, Blatterer, Berger & Kohmann 1991	-	-	-	-	-	+	-
Colpoda formisanoi Foissner, Agatha & Berger 2002	-	-	-	+	-	-	-
Colpoda inflata (Stokes 1884) Kahl 1931	+	+	+	+	+	+	+
Colpoda maupasi Enriques 1908	+	+	+	+	+	+	+
Colpoda steinii Maupas 1883	+	+	+	+	+	+	+
Cultellothrix atypica (Wenzel 1953) Foissner & Xu 2007	-	+	-	-	-	-	-
Cyrtolophosis acuta Kahl 1926	+	-	+	+	-	+	+
Cyrtolophosis minor Vuxanovici 1963	+	-	-	+	-	-	-
Cyrtolophosis mucicola Stokes 1885	+	+	+	+	+	+	+
Edaphospathula brachycaryon Foissner & Xu 2007	-	-	+	-	-	-	-

Edaphospathula fusioplites (Foissner, Berger, Xu & Zeichmeister-Boltenstern 2005) Foissner & Xu 2007	+	-	-	-	+	+	-
Enchelys terrenum (Foissner 1984) Vďačný 2007	+	-	+	-	-	-	-
Exocolpoda augustini (Foissner 1987) Foissner, Agatha & Berger 2002	+	+	+	+	-	-	+
Fuscheria terricola Berger & Foissner 2007	+	+	+	+	-	-	+
Gastrostyla sp.	-	-	-	-	+	-	-
Gonostomum affine (Stein 1859) Sterki 1878	+	+	+	+	+	+	+
Gonostomum strenuum (ss)[a] (Engelmann 1862) Sterki 1878	+	+	+	+	-	-	+
Gonostomum strenuum (V)[b]	+	-	+	+	-	-	+
Grossglockneria acuta Foissner 1980	+	+	+	+	+	+	+
Grossglockneria hyalina Foissner 1985	+	+	+	+	-	-	+
Haptorid taxon 1	-	-	-	-	-	-	+
Haptorid taxon 2	-	-	-	-	-	-	+
Hausmaniella discoidea (Gellért 1956) Foissner 1984	+	-	+	-	-	-	-
Hemisincirra sp.	-	-	-	-	+	+	-
Hemisincirra inquieta Hemberger 1985	+	+	+	+	-	+	+
Hemisincirra interrupta (Foissner 1982) Foissner in Berger 2001	+	+	+	-	+	-	+
Hemisincirra quadrinucleata Hemberger 1985	+	+	+	-	+	+	+
Hemisincirra wenzeli Foissner 1987	-	-	-	-	-	+	-
Homalogastra setosa Kahl 1926	+	+	+	+	+	-	+
Lagynophrya sp.	-	-	+	-	-	-	-
Lamtostyla islandica Berger & Foissner 1988	+	-	-	-	-	+	-
Lamtostyla perisincirra (Hemberger 1985) Berger & Foissner 1987	+	-	+	-	-	+	-
Leptopharynx (costatus) costatus Mermod 1914	+	+	+	+	+	+	+
Microdiaphonasoma arcuatum Wenzel 1953	+	-	+	-	-	-	-
Nivaliella plana Foissner 1980	+	-	+	+	-	-	+
Notoxoma parabryophryoides Foissner 1993	-	+	+	-	-	+	+
Odontochlamys alpestris Foissner 1982	+	+	+	-	-	+	-

Odontochlamys (alpestris) biciliata Foissner, Agatha & Berger 2002	-	+	-	-	-	-	-
Ottowphrya dragescoi (Foissner 1987) Foissner, Agatha & Berger 2002	+	+	-	-	-	-	+
Parabryophrya penardi (Kahl 1931) Foissner 1985	-	-	-	+	-	-	-
Parabryophrya terricola Foissner 1985	-	-	+	+	-	-	-
Paraenchelys spiralis Foissner 1983	-	-	-	+	-	-	-
Paraenchelys terricola Foissner 1984	+	+	+	+	+	-	-
Paragastrostyla sp.	+	-	-	-	-	-	-
Paragastrostyla lanceolata Hemberger 1985	-	-	+	-	-	-	-
Paraholosticha muscicola (Kahl 1932) Wenzel 1953	+	+	-	-	-	-	-
Periholosticha lanceolata Hemberger 1985	+	+	+	+	-	-	+
Periholosticha paucicirrata Foissner, Berger, Xu & Zeichmeister-Boltenstern 2005	+	-	-	-	-	-	-
Periholosticha silvatica Foissner, Berger, Xu & Zeichmeister-Boltenstern 2005	+	-	-	-	-	-	-
Plagiocampa ovate Gelei 1954	+	+	+	+	-	-	+
Platyophrya binucleata Foissner 1987	+	+	+	-	-	-	-
Platyophrya macrostoma Foissner 1980	-	+	+	-	-	-	-
Platyophrya spumacola Kahl 1927	-	-	-	-	+	-	-
Platyophrya vorax Kahl 1926	+	+	+	+	+	+	+
Plesiocaryon elongata (Schewiakoff 1892) Foissner, Agatha & Berger 2002	-	-	-	-	-	-	+
Protocyclidium muscicola (Kahl 1931) Foissner, Agatha & Berger 2002	+	+	+	+	+	-	+
Protospathidium serpens (Kahl 1930) Foissner 1981	+	+	-	+	-	+	+
Protospathidium vermiforme Foissner, Agatha & Berger 2002	-	-	-	+	-	-	+
Pseudochilodinopsis fluviatilis Foissner 1988	+	+	+	+	-	+	-
Pseudocyrtolophosis terricola Foissner 1993	+	+	+	+	-	-	+
Pseudomonilicaryon breviproboscis	+	-	-	-	-	-	-
Pseudoplatyophrya nana (Kahl 1926) Foissner 1980	+	+	+	+	+	+	+
Pseudoplatyophrya saltans Foissner 1988	+	+	+	+	-	-	+
Sathrophilus muscorum (Kahl 1931) Corliss 1960	+	-	+	-	-	-	-
Spathidiid taxon	+	-	-	-	-	-	-

Spathidium sp. Dujardin 1841	-	-	+	+	-	-	-
Spathidium spathula (Müller 1773) Moody 1912	-	-	+	-	-	-	-
Trihymena terricola Foissner 1988	+	+	+	+	-	-	-
Urosoma acuminata (Stokes 1887) Bütschli 1889	+	-	-	-	-	-	-
Urosomoida agiliformis Foissner 1982	+	-	+	-	-	-	-
Vorticellides astyliformis (Foissner 1981) Foissner, Blake, Wolf, Breiner & Stefánsson 2009	+	+	+	+	-	-	-
Number of species	58	41	53	40	21	24	37
	62		60			52	

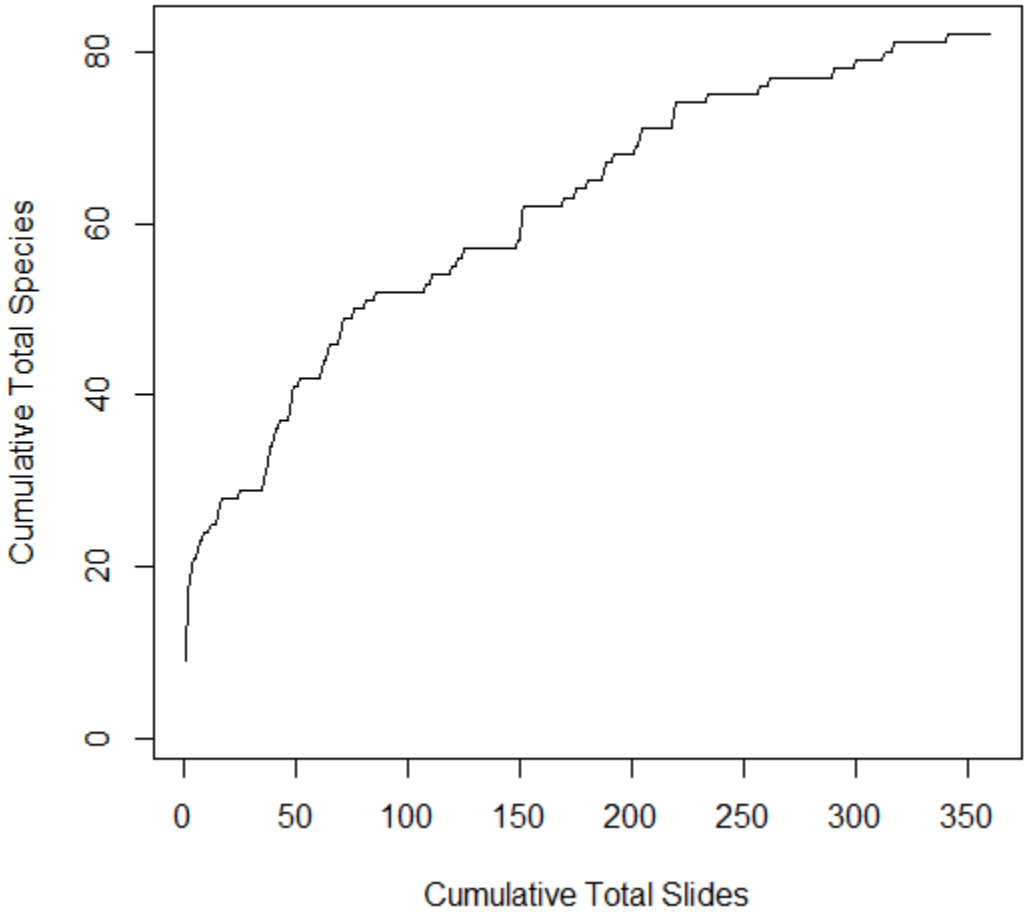

Fig. 5. Species accumulation curve based on all slides examined across 6 plots (a total of 7 samples). With each new slide, species are still being added.

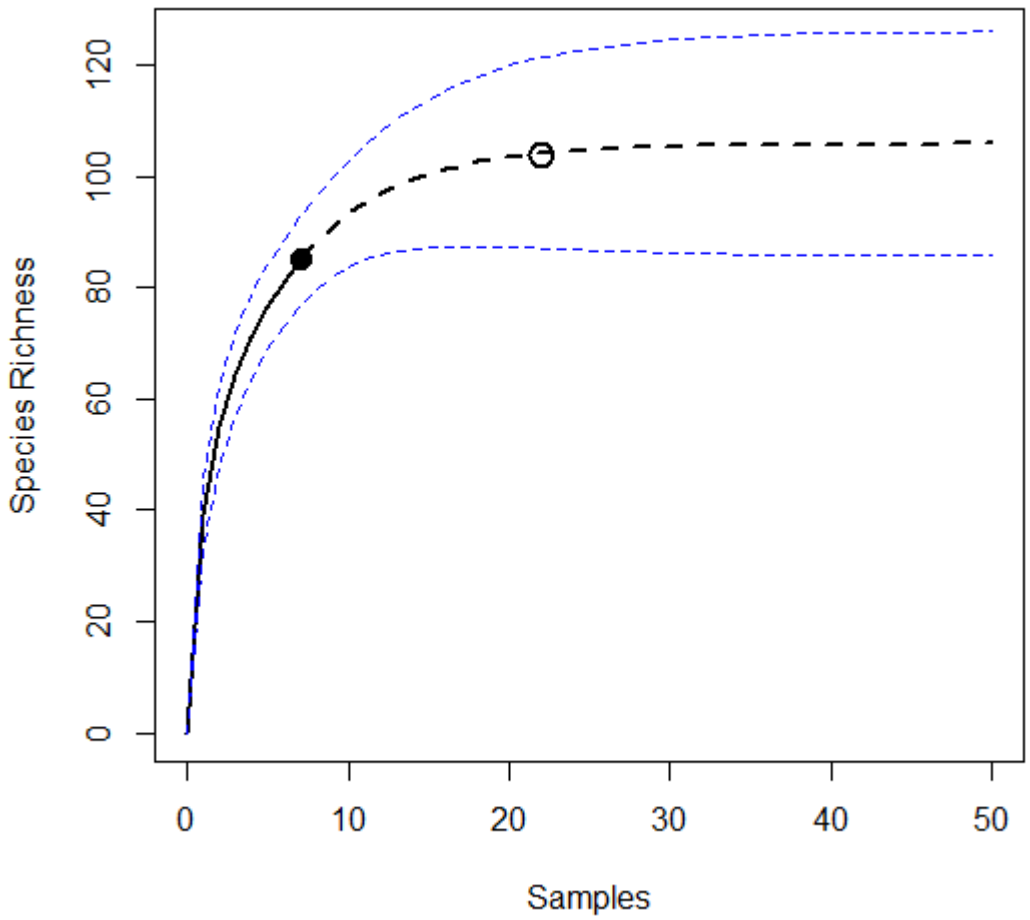

Fig. 6. Rarefaction curve from sampling effort across seven samples. Solid line is rarefaction curve; solid circle is the depth of actual sampling; dashed black line is curve extrapolation, based on EstimateS algorithms using a Bernoulli product model (Colwell et al. 2012). Open circle is Chao2 estimate of richness. Dotted blue lines are 95% CI.

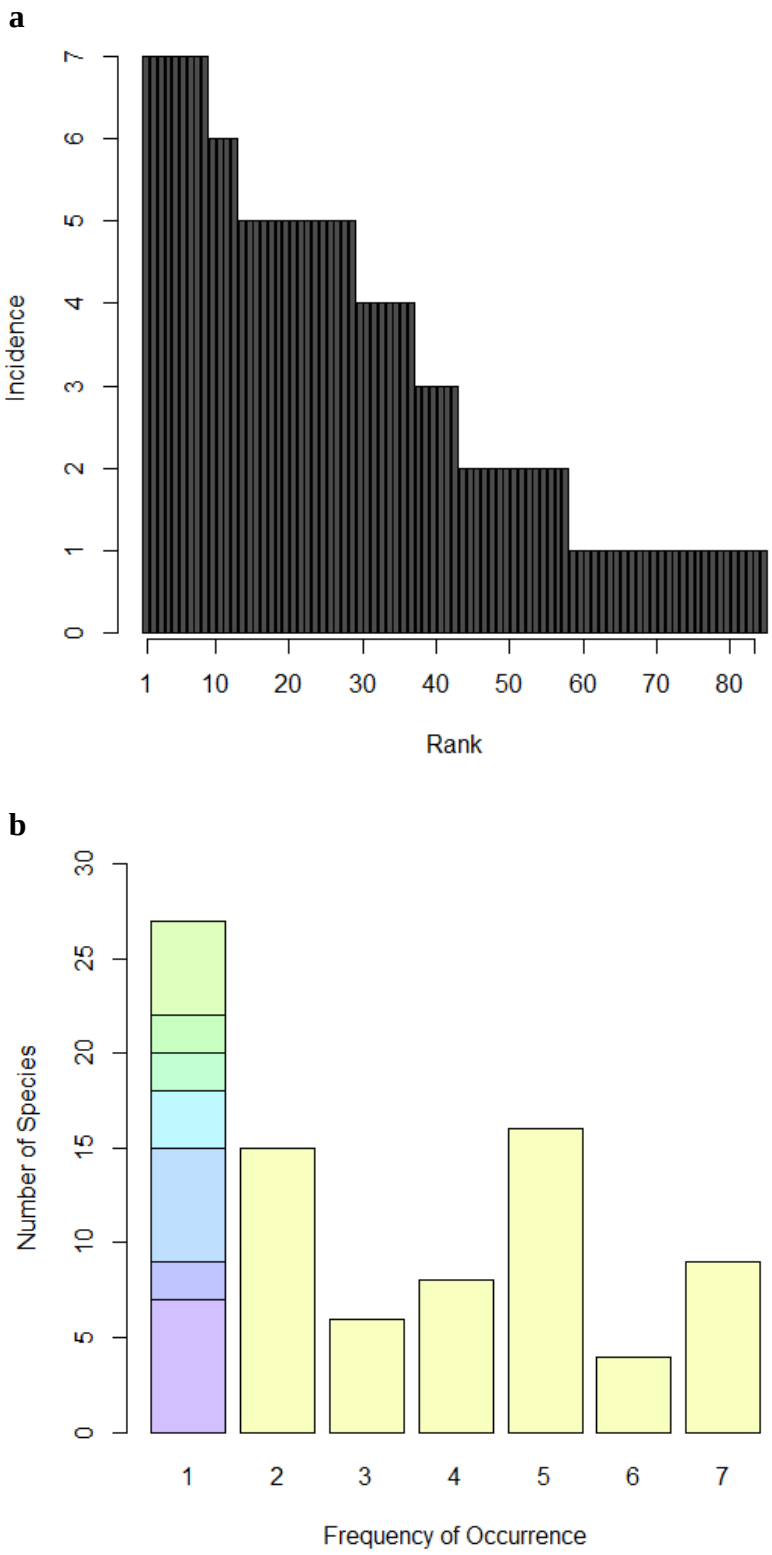

Fig. 7. (a) Rank-incidence curve based on sampling effort across seven samples. (b) Frequency with which species were encountered. Divisions in first bar indicate from which samples uniques were observed (bottom to top: Au, Ab, Bu, Bb, Cu, Cb, Cb2). Remaining bars indicate number of species seen in more than one sample.

Table 3. Taxonomic classification of the ciliate community found in samples of sagebrush associated soils of southwestern Idaho (SASI), compared to global populations and a study conducted in decaying wood moss (DWM) of Slovakia. (Adapted from Bartošová and Tirjaková 2008, with permission from *Acta Protozoologica*)

Class	Global[a]		DWM[b]		SASI	
	Species	%	Species	%	Species	%
Colpodea	129	21.8	23	18.9	31	35.7
Phyllopharyngea	26	4.2	4	3.3	3	3.6
Litostomatea	105	17.8	39	32	19	22.6
Spirotrichea	215	36.1	36	29.5	25	29.8
Oligohymenophorea	58	9.9	13	10.7	4	4.8
Nassophorea	26	4.4	5	4.1	1	1.2
Prostomatea	10	1.7	0	0	1	1.2
Heterotrichea	24	4.1	2	1.6	1	1.2
Total	593	100	122	100	85	100
Samples/replicates	817		154		7	

[a] = Foissner (1998)
[b] = Bartošová and Tirjaková (2008)

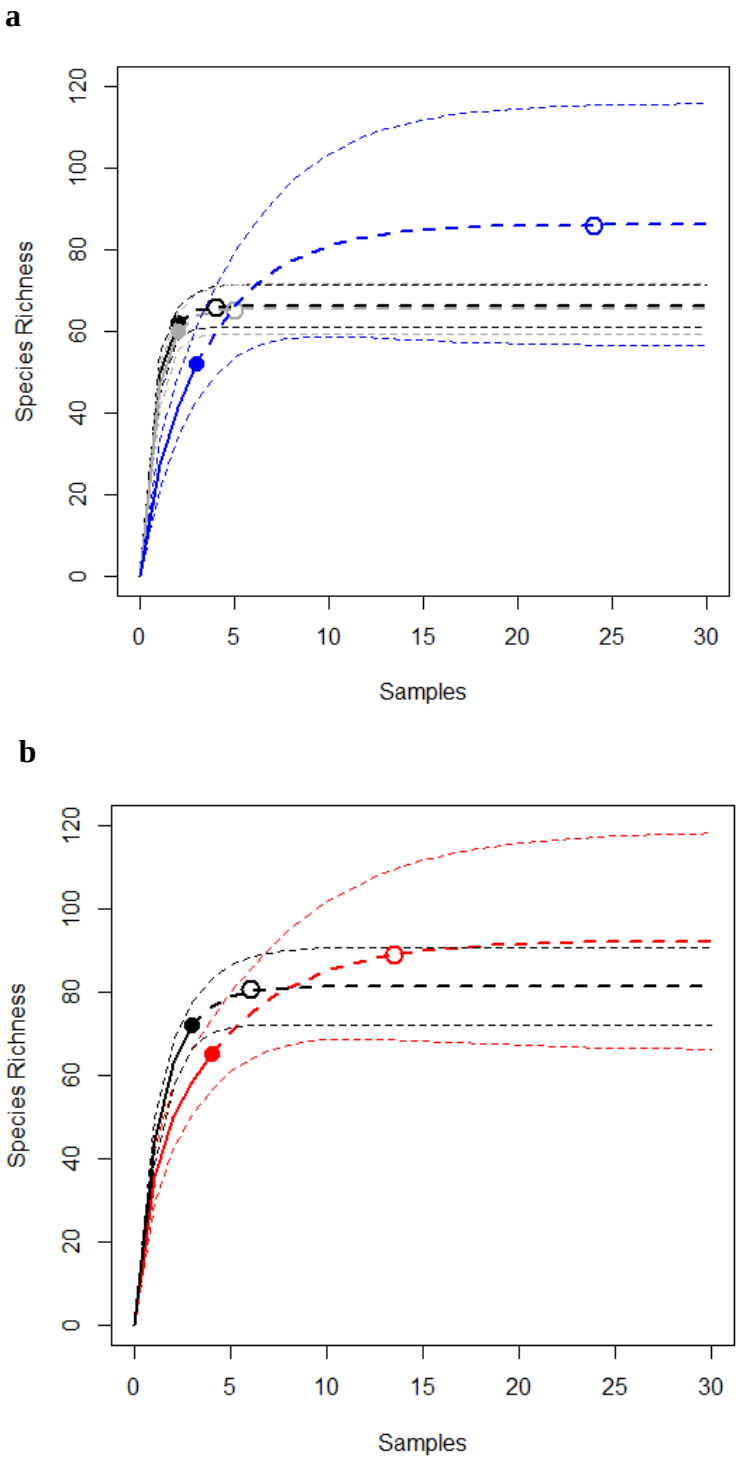

Fig. 8. Rarefaction curves by site (a) and plot condition (b). (Site A=black, B=grey, C=blue; burned plots=red, unburned=black.) Solid lines are rarefaction curves; solid circles are depth of actual sampling; dashed lines extending from solid circles are curve extrapolations, based on EstimateS algorithms using a Bernoulli product model. Open circles are Chao2 estimates of richness. 95% CI are shown as dashed lines on either side of curves.

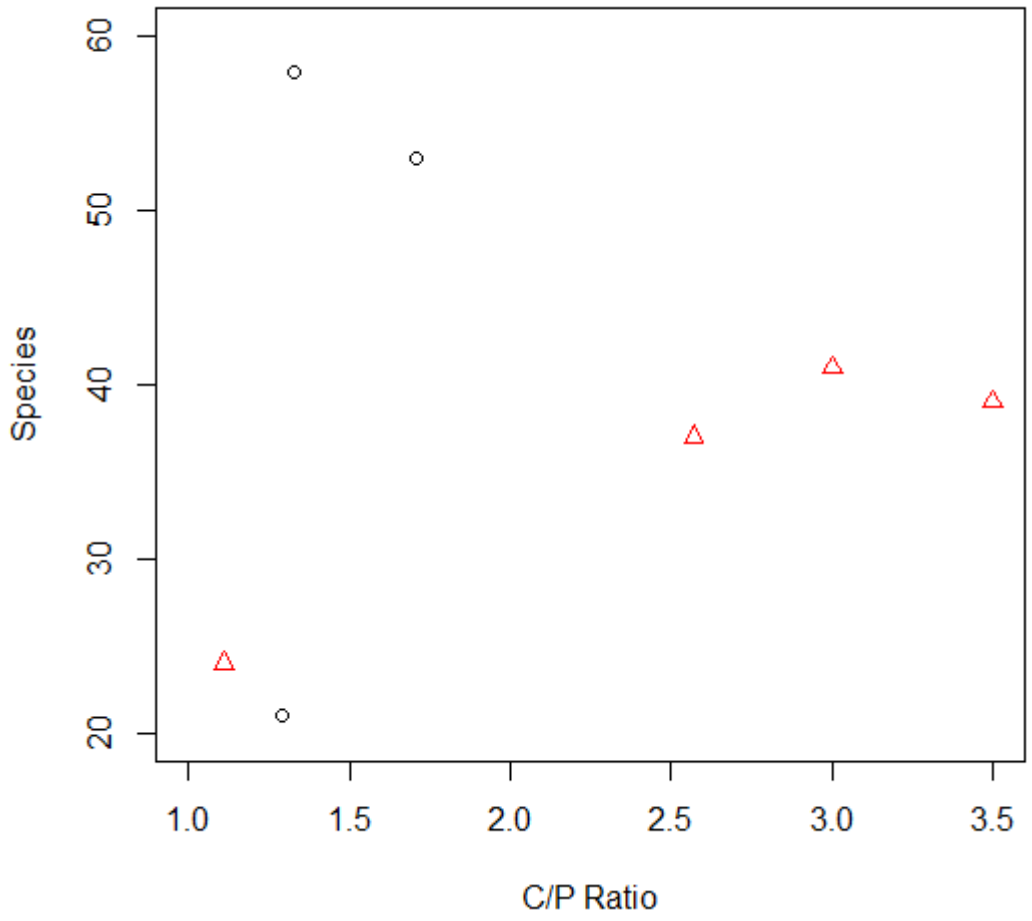

Fig. 9. Relationship of C/P ratio to species number by plot condition across seven samples. Black circles represent unburned replicates, and red triangles represent burned sample values.

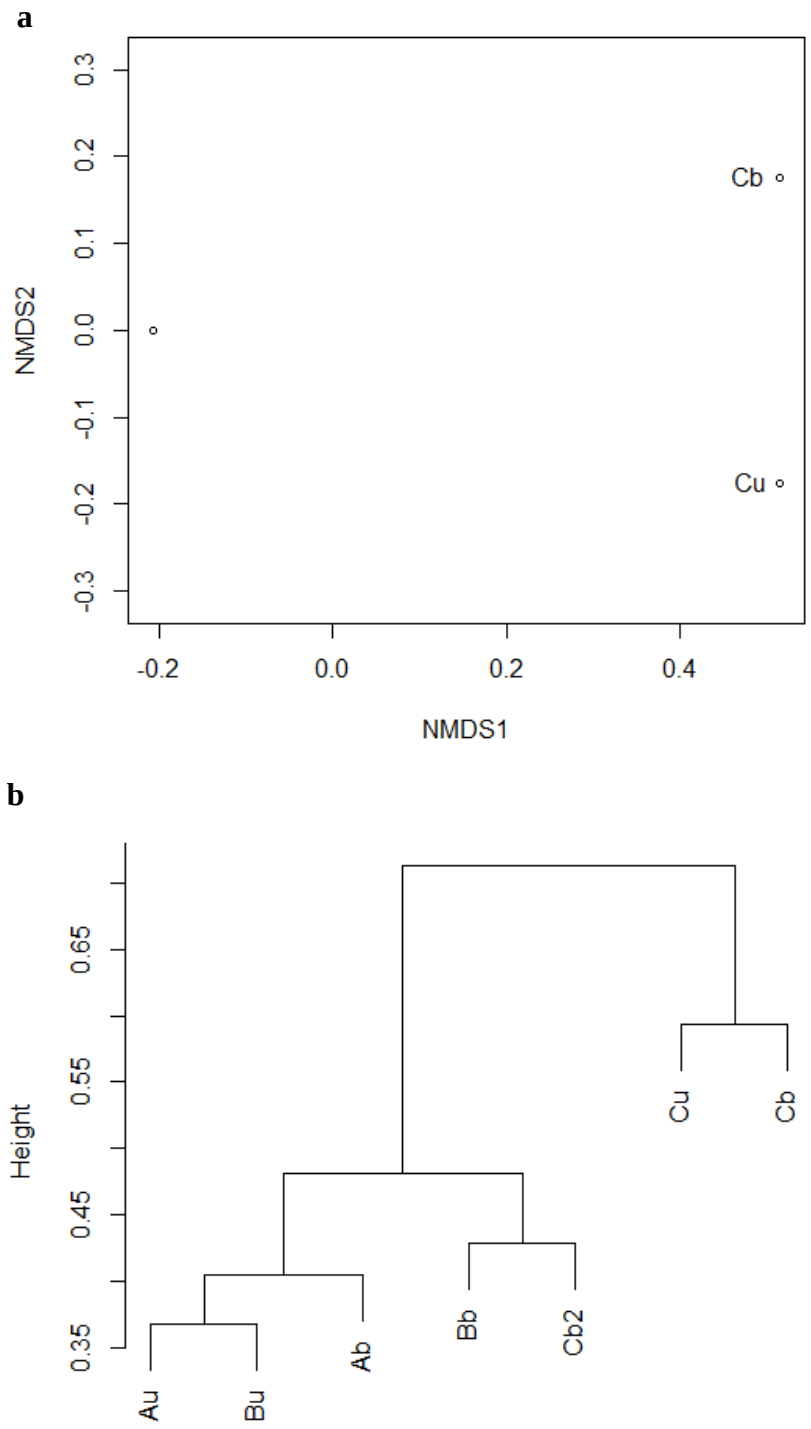

Fig. 10. (a) Non-metric multidimensional scaling (NMDS) and (b) unweighted pair-group arithmetic mean (UPGMA) analyses. All points in (a) are clustered at the same coordinates, except for the points from replicate Cu and its pair, Cb (labeled). Axes are ordinated; differences are seen on NMDS1 and NMDS2. Replicates Cu and Cb cluster together in the UPGMA analysis, separate from all others. Bb and Cb2 form a second cluster, and Au, Bu, and Ab form the third.

Table 4. Summary of soil analysis parameters (a) by plot (5 sampling locations each) and (b) by sample used for ciliate analysis. Values in parentheses are standard deviations.

a

Plot	%C	%N	pH	% Clay	Soil Texture
Au	2.89	0.17	6.49 *(0.21)*	14.1 *(1.7)*	Sandy loam
Ab	3.30	0.17	6.62 *(0.23)*	6.0 *(2.6)*	Sandy loam
Bu	3.49	0.25	6.73 *(0.51)*	9.6 *(2.6)*	Sandy loam
Bb	5.66	0.35	6.66 *(0.22)*	10.3 *(3.6)*	Sandy loam
Cu	4.33	0.36	7.11 *(0.58)*	10.7 *(2.8)*	Loam
Cb	4.90	0.40	7.31 *(0.12)*	11.0 *(1.1)*	Loam

b

Sample	pH	% Clay	Soil Texture
Au	6.3	15.5	Sandy loam
Ab	6.96	2.5	Fine Sand
Bu	7.56	10.5	Sandy loam
Bb	6.81	5.9	Loamy fine sand
Cu	6.97	14.6	Sandy loam
Cb	7.44	10	Loam
Cb2	7.39	9.7	Loam

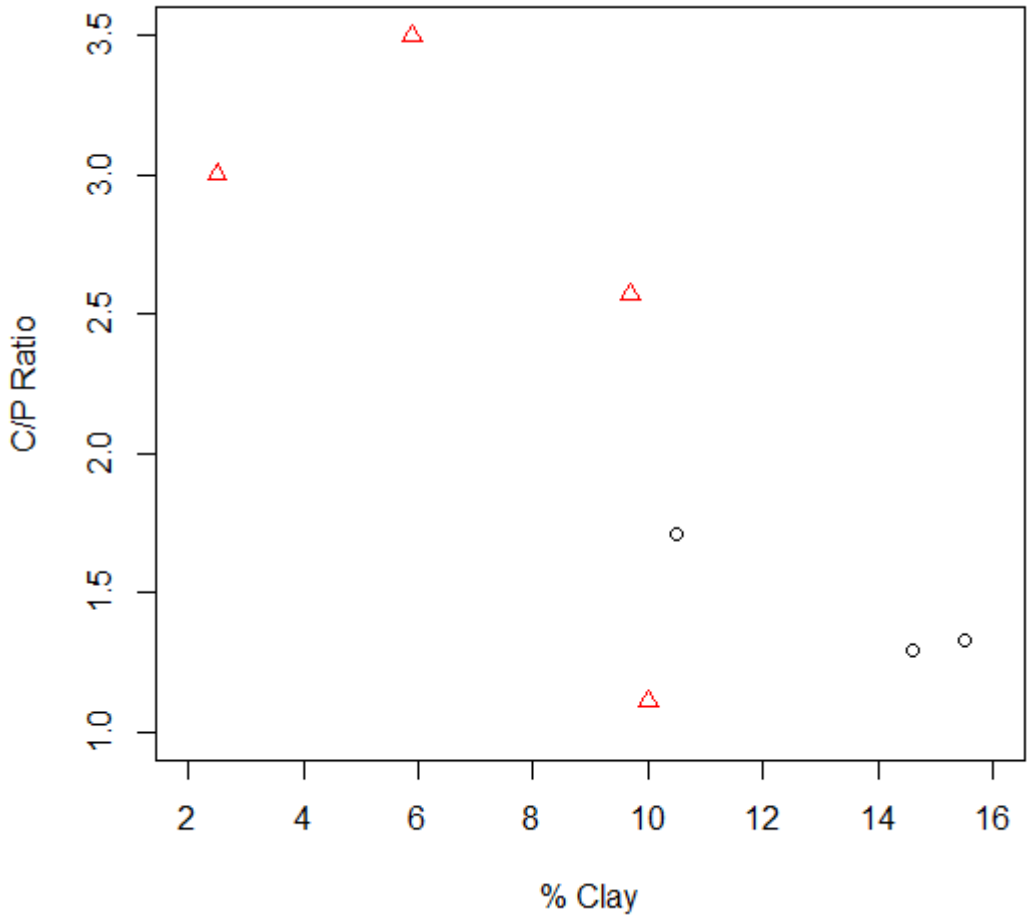

Fig. 11. Association of percent clay and the corresponding C/P ratio, as detected from each sample. Black circles represent unburned samples, and red triangles represent burned sample values.

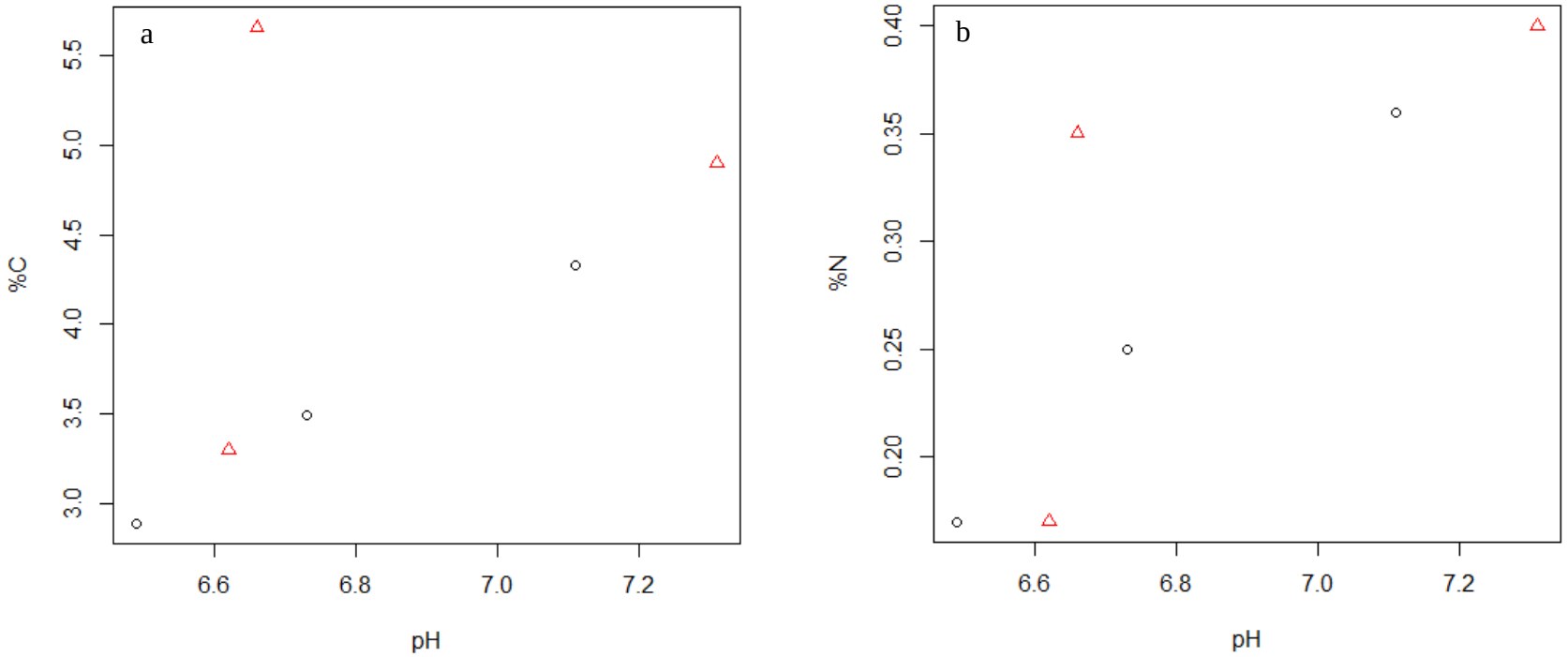

Fig. 12. **Relationship of pH to % C (a) and pH to % N (b), by plot condition. Black circles represent unburned plots, and red triangles represent burned plot values.**

APPENDIX A

Method for Fixing Permanent Slides and High Flow-through Staining

Procedure:

Modified from Foissner (1991), Dieckmann (1995), Bourland (pers. comm.), and personal observations

1. Add 5 ml Bouin's Fixative to a 15 ml centrifuge tube.

2. Gently tilt non-flooded petri dish 45º. Using a transfer pipette, remove 5 mL of fluid by sweeping the pipette along the edge of the dish where the percolate collects, continuing to rotate the dish. Aspirate gently from the soil surface to collect any material from the center of the dish. Add to tube with fixative.

3. Cap centrifuge tube and vigorously shake. Let material sit at room temp for 15 min. to allow for complete fixation. Centrifuge for 30 s at 2000 rpm to concentrate cells at bottom of tube.

4. Bouin's fluid must now be washed out of solution Carefully decant 8.5 mls of the supernatant with a transfer pipette, leaving 1.5 mls in the bottom of the tube. Add tap water to 10 mls and gently shake. Centrifuge this again for 30 s at 2000 rpm. Repeat washes until color of liquid in tube is clear and not yellow (may take 6-7 washes).

5. After last wash, decant to 1.5 mls and fill with DI H_2O to 5 mls. Add two drops of Mayer's Albumin into the tube, cap, and vigorously shake. Foam should develop at the liquid-air interface. Add more DI to 10 mls, cap, and vigorously shake again. Leave mixture at room temp for 40 min to allow enrobement of fixed ciliates in albumin.

6. Prepare 8-16 slides and label each. To clean surface of slide, firmly wipe a lint-free tissue with ethanol across the surface of each slide, ensuring that the entire slide (except for the frosted edge) has been cleaned. Flame-dry each one to evaporate alcohol. When done, place them on a flat surface, and cover until use.

7. Centrifuge the albumin-ciliate mixture for 30 s at 2000 rpm Decant the supernatant by pipette into waste, leaving 1.5 mls.

8. Vigorously agitate the remaining material with a transfer pipette. Using all 1.5 ml, pull up a drop at a time, varying the size, and place on slides. After each transfer, use a mounting needle to evenly spread the material across the entire surface of the slide. All slides should remain flat and covered until material dries. Slides may be stained with protargol immediately, or stored appropriately for future staining.

9. To begin high through-put staining, place slides back to back in a vertical staining jar filled with 95% alcohol for 20 min. Place staining jar with 0.4-0.8% protargol solution in 60 C oven to allow sufficient time to warm.

10. Rehydrate slides through 70% alcohol and two tap water washes for 5 minutes each.

11. Place slides in 0.2% potassium permanganate solution for 2 min. Collect slides in staining jar filled with tap. Quickly discard tap while slides are in there, and add fresh down side.

12. Immediately transfer slides to 2.5% oxalic acid for 3 min. Collect slides in staining jar filled with tap water.

13. Immerse slides in tap water two times for 3 min each, and once in DI for 3 min.

14. Place slides in warm protargol for 15-30 min (up to 1 hour).

15. Remove staining jar with the slides from the oven and take one slide from the mid series for adjusting the developer, and place on a flat surface. Flood slide with protargol from the staining jar, followed by two drops of acetone developer. Using a pipette tip, make sure the solution covers the entire slide; start the timer and swirl to mix. Slides may take 30 s to 20 min to develop, so check repeatedly by pouring off excess solution, adding a coverslip, and checking development under microscope. If overdeveloped or perfectly developed, pull out rest of slides and bracket the time for developing (some ciliates will stain better with more or less time). If underdeveloped, return slide to tray, remove coverslip, add more protargol and developer, and recheck in a few minutes. Continue this process until optimal development is reached.

16. Once each slide has developed, remove excess protargol and developer. Dip slide twice briefly into beaker filled with tap water, and once into a beaker filled with DI.

17. Fix slides in 2.5% sodium thiosulphate for 3 s. Immerse in tap water 3 times for 3 min each.

18. Transfer slides through alcohol series for dehydration (70%, 100%, 100% ethanol) for 3-5 min each.

19. Add 1-2 drops Euparal onto slide. Place coverslip on, making sure to avoid bubbles. Leave slides flat, face up and covered for 24-48 hours to dry.

<u>Reagents:</u>

1. Bouin’s Fixative (purchase commercially; 5 ml required for each sample)

2. Mayer's Albumin *from Foissner 1991*
   Pour 3 free-range egg whites into sterile polycarbonate 250 mL flask; cap and shake vigorously until no more foam develops. Let flask sit for 1 min; should see separation of liquid and foam. Pour liquid portion into a second sterile flask; repeat treatment from first flask. Transfer liquid through about 6 flasks- let all flasks then sit for about ten minutes. A watery fluid should appear from the foam at the bottom of each of the flasks (do not use the material from the last flask). Pour this into a sterile container and add an equal amount of 98-100% glycerol. Finally, add a crystal of thymol for preservation. Store in refrigerator. If whitish film develops at the bottom of the container, can decant liquid portion into new sterile container. Will remain stable for several months.

3. 0.4-0.8% protargol (made in house, see Pan et al. 2013)
   May be reused up to three series of slide transfers, but never more than 12 hours
   0.024-0.48 g in 60 mL DI

4. 0.2% potassium permanganate
   May be reused up to three series of slide transfers, but never more than 12 hours
   0.12 g dissolved in 60 ml DI

5. 2.5% oxalic acid
   May be reused up to three series of slide transfers, but never more than 12 hours
   1.5 g dissolved in 60 mL DI

6. 2.5% sodium thiosulphate
   25 g in 1 L (stable for years)

7. Acetone Developer (add in the following order, stable for 2 weeks, though impregnation time may take substantially longer the older the developer)
   40 mL DI
   0.7 g boric acid
   0.15 g hydroquinone
   1 g sodium sulphite
   7.5 mL acetone

APPENDIX B

Selected Images of Observed Ciliates

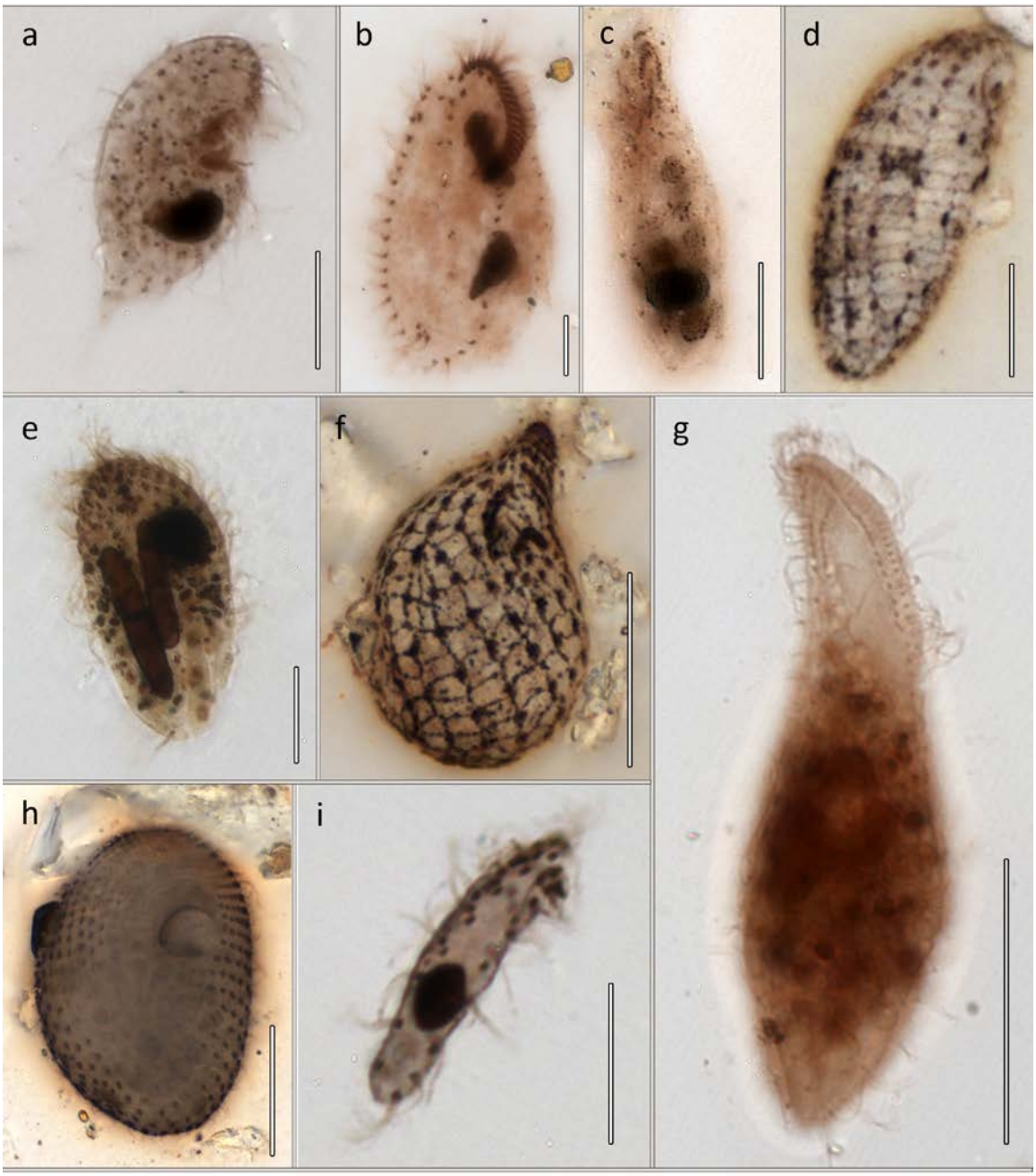

Fig. B1. Selected ciliates from SASI soils showing variation and diversity of species after protargol impregnation (a-c, e, g, i) and Chatton-Lwoff silver nitrate impregnation (d, f, h). a) *Colpoda formisanoi;* b) *Gonostomum strenuum;* c) *Trihymena terricola;* d) *Grossglockneria hyalina;* e) *Avastina ludwigi;* f) *Exocolpoda augustini;* g) *Arcuospathidium (muscorum) muscorum*; h) *Hausmaniella discoidea;* i) *Pseudoplatyophrya nana.* Scale bars: 10 µm (a-e, i), 25 µm (f-h).