Available online at www.sciencedirect.com

ScienceDirect

Applied Soil Ecology 35 (2007) 622–634

Applied Soil Ecology

www.elsevier.com/locate/apsoil

Assessing exotic plant species invasions and associated soil characteristics: A case study in eastern Rocky Mountain National Park, Colorado, USA, using the pixel nested plot design

Mohammed A. Kalkhan [a,*], Evan J. Stafford [a], Peter J. Woodly [a], Thomas J. Stohlgren [b]

[a] *Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523-1499, USA*
[b] *National Institute of Invasive Species Science, Fort Collins Center, U.S. Geological Survey, 2150 Center Ave., Fort Collins, CO 80526, USA*

Received 16 December 2005; received in revised form 23 August 2006; accepted 5 September 2006

Abstract

Rocky Mountain National Park (RMNP), Colorado, USA, contains a diversity of plant species. However, many exotic plant species have become established, potentially impacting the structure and function of native plant communities. Our goal was to quantify patterns of exotic plant species in relation to native plant species, soil characteristics, and other abiotic factors that may indicate or predict their establishment and success. Our research approach for field data collection was based on a field plot design called the pixel nested plot. The pixel nested plot provides a link to multi-phase and multi-scale spatial modeling-mapping techniques that can be used to estimate total species richness and patterns of plant diversity at finer landscape scales. Within the eastern region of RMNP, in an area of approximately 35,000 ha, we established a total of 60 pixel nested plots in 9 vegetation types. We used canonical correspondence analysis (CCA) and multiple linear regressions to quantify relationships between soil characteristics and native and exotic plant species richness and cover. We also used linear correlation, spatial autocorrelation and cross correlation statistics to test for the spatial patterns of variables of interest. CCA showed that exotic species were significantly ($P < 0.05$) associated with photosynthetically active radiation ($r = 0.55$), soil nitrogen ($r = 0.58$) and bare ground ($r = -0.66$). Pearson's correlation statistic showed significant linear relationships between exotic species, organic carbon, soil nitrogen, and bare ground. While spatial autocorrelations indicated that our 60 pixel nested plots were spatially independent, the cross correlation statistics indicated that exotic plant species were spatially associated with bare ground, in general, exotic plant species were most abundant in areas of high native species richness. This indicates that resource managers should focus on the protection of relatively rare native rich sites with little canopy cover, and fertile soils. Using the pixel nested plot approach for data collection can facilitate the ecological monitoring of these vulnerable areas at the landscape scale in a time- and cost-effective manner.

Keywords: Exotic plant species; Plant diversity; Rapid assessment; Pixel nested plot; Landscape scale; Geospatial information; Canonical correspondence analysis; Geostatistics

---

* Corresponding author. Tel.: +1 970 491 5262; fax: +1 970 491 1965.
E-mail address: mohammed@nrel.colostate.edu (M.A. Kalkhan).

0929-1393/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2006.09.009

1. Introduction

Rocky Mountain National Park (RMNP) contains a diversity of plant species. Though many of the plant species in the park are native, there are many harmful exotic species that, by definition, invade native vegetation. The RMNP has worked to control the invasion of exotic plant species since 1960 (Short, 1987). Part of our overall project goal was to evaluate the degree and severity of invasions. The locations in which these exotic plant species are found are dependent on a combination of variables, such as altitude, watershed orientation, aspect, soil development, proximity to trails and road networks, and history of disturbance.

Usually, to quantify the relationship between variables and plant species, researchers are forced to limit the number of variables which they can investigate. In our study, we attempt to quantify these factors using multivariate and geostatistic methods to examine the associations of each species to specific environmental variables. By linking the presence of plant species, both native and exotic, with soil characteristics and other abiotic factors (light interception, aspect, slope) we can develop predictive multivariate statistical models to quantify patterns of plant diversity, species richness, invasion of exotic species, and other biotic variables.

Multivariate statistics are commonly used in forecasting ecological and environmental parameters. With the improved speed of computing technology, researchers are becoming more aware of the benefits of also using geospatial statistics. In this study, we used canonical correspondence analysis (CCA, see ter Braak, 1986, 1987; Palmer, 1993; Reed et al., 1993; Bashkin et al., 2003), linear correlation, spatial autocorrelation, and cross-correlation statistics (Moran, 1948; Geary, 1954; Fortin et al., 1989; Bonham et al., 1995; Reich et al., 1995; Kalkhan and Stohlgren, 2000) as tools to understand the spatial relationships between biotic and abiotic variables of interest, and to identify variables related to the presence of native versus exotic plant species. These statistical tools then form the foundation for developing geospatial models and thematic maps of natural resources and environmental gradients. For example, Kalkhan and Stohlgren (2000) found that spatial autocorrelation and cross-correlation statistics can be used to evaluate spatially explicit information on vegetation characteristics and structures (e.g., plant cover-abundance, patterns of old-growth forest stands, and plant species richness patterns), and associated environmental characteristics (e.g., topographic, edaphic, and resource availability).

Collecting field data and selecting a proper plot configuration play an important role in biotic and abiotic surveys. They enable researchers and resource managers to rapidly assess and therefore better understand the spatial patterns at which biotic and abiotic variables occupy the landscape. We used a 15 m × 15 m pixel nested plot (PNP) design (Kalkhan, 2005) to rapidly quantify soil parameters and to relate these factors to the environmental and geographical relationships of particular plant species. The 15 m × 15 m plot size was employed to make a rapid assessment possible in the challenging terrain of RMNP. The PNP field sampling approach is linked to geospatial information data (remote sensing, geographic information systems (GIS), and global positioning system (GPS)) formats allowing a rapid and efficient application to multi-scale geospatial modeling techniques that are used to estimate total species richness and patterns of plant diversity at landscape scales.

Rectangular (MWNP: Modified-Whittaker Nested Plot, Stohlgren et al., 1995), circular (FHM: Forest Health Monitoring, USDA Forest Service, and the 500 m^2: Kirkman et al., 2000), systematic grid-circular (FIA: Forest Inventory Analysis, USDA Forest Service, http://www.fia.fs.fed.us/tools-data/), and linear (Planar Intersect, Parker, 1951; Daubenmire, 1959) plot shapes are the more common field inventory approaches and are useful for analyzing vegetation, biotic, and abiotic characteristics, however these plot types are sometimes hard to link to geospatial information data (Kalkhan, 2005). In comparison to the plots listed above, the pixel nested plots use random Universal Transverse Mercator (UTM) coordinates as the plot's center point. In addition, the dimensions of the plot's square design allows for the maximized use of the information provided by averaging spectral reflectance values of the remote sensing pixels (see ESRI, 1999). If the goal of the researcher is to develop a geospatial model and thematic map of the landscape, the representation of a remotely sensed pixel by each PNP can assist in creating a transition link between the field plots and the remotely sensed pixels (Kalkhan, 2005).

In contrast to the earlier studies using square plots at various sizes ranging from very small to very large (Smith et al., 1986, 1987; Metzger, 1997; Townsend and Walsh, 2001; Joy et al., 2003; Reich et al., 2004; Woldendorp et al., 2004; Woodall and Graham, 2004, and the USGS-National Park Service Vegetation Mapping Program using a 20 m × 20 m square plot design; see http://biology.usgs.gov/npsveg/standards.html), the pixel nested plot (PNP) samples biotic and abiotic characteristics at fine scale resolution (1–15 m^2). Since there is a need to measure patterns of diversity and other ecological variables at finer scales to create a direct link to the geospatial information and thematic mapping applications, this data can be used to better extrapolate the landscape from fine to coarse scales when the square plot layout is used. The PNP accommodates this need for forecasting ecological and environmental parameters and in particular, patterns of plant species invasions.

Rocky Mountain National Park's establishment acknowledges native plant populations and their importance in the historical and future ecological balance. Exotic plant species invasion will change the ecological balance and threatens the characteristics that define the park, such as critical animal habitat, rare plant communities, and undisturbed wilderness. Exotic plants species (e.g., leafy spurge) can locally replace native plant species and can be toxic to wildlife or livestock (Harper et al., 1996; Brown, 2003). Stohlgren et al. (2003) showed that exotic plant species have successfully invaded areas rich in native plant diversity such as the tallgrass prairie, wet meadows, and aspen vegetation types in the Rocky Mountains and central grasslands (Stohlgren et al., 1999, 2001). In some of the areas of the park, exotic plant species are widely dispersed and in many areas may become locally dominant. Our interest was whether exotic plant species invasion was correlated with particular soil characteristics. We used vegetation and soils survey data from a new sampling plot design, the pixel nested plot (PNP), and a correlative approach to examine specific soil parameters under which exotic plants have been successfully established in Rocky Mountain National Park, Colorado, USA. Our objective was to quantify patterns of native and exotic plant species richness relative to certain abiotic soil characteristics at the landscape-scale using an alternative sampling plot design (PNP), canonical correspondence analysis, linear correlation, spatial autocorrelation, and cross correlation statistics.

2. Materials and methods

2.1. Study area

Rocky Mountain National Park, Colorado, USA (Fig. 1) is located in the northern Front Range of Colorado and covers an area of approximately 107,500 ha and ranges in elevation between 2100 and

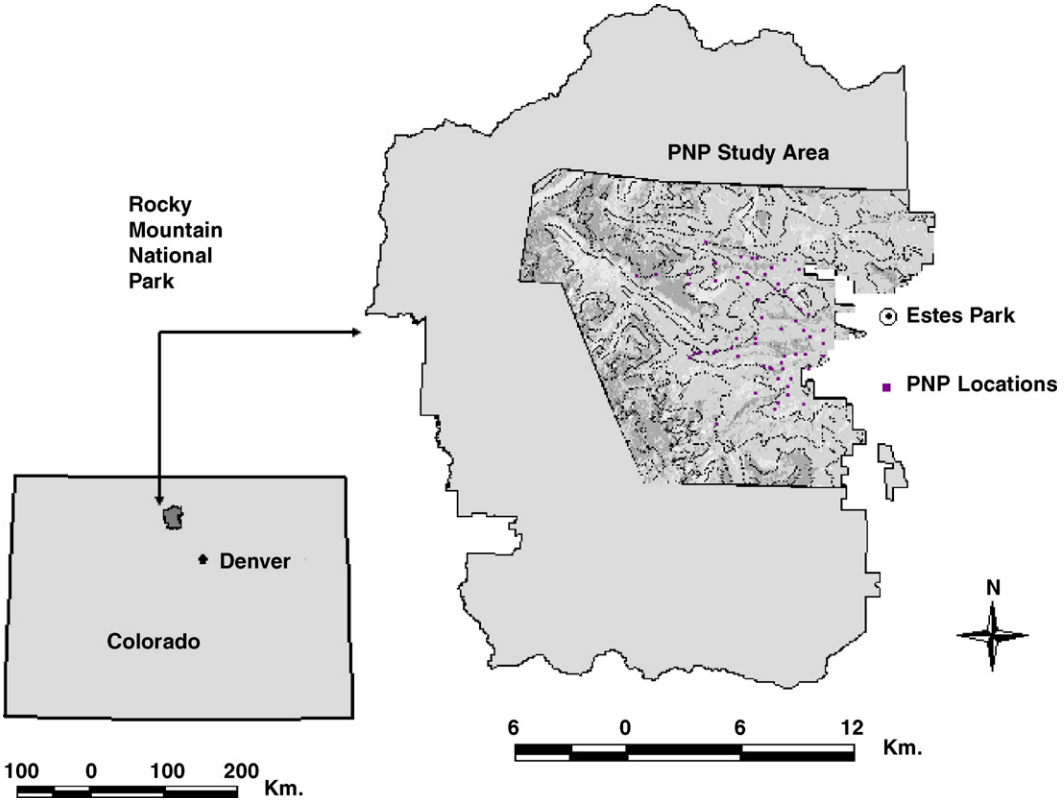

Fig. 1. Map of the pixel nested plot (60 PNP) study area within Rocky Mountain National Park, CO, USA.

4300 m. Our study area consisted of an area of approximately 35,000 ha (Fig. 1), located on the eastern side of the Continental Divide, between Loch Vale watershed to the south and Bighorn Mountain to the north. The site ranges in elevation between 2300 and 4000 m and is dominated by five general vegetation communities: meadow, ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), spruce/fir (*Picea engelmannii*, *Abies lasiocarpa*), Douglas-fir (*Pseudotsuga menziesii*), and alpine. This area also contains relatively rare communities such as riparian, aspen (*Populus tremuloides*), and limber pine (*Pinusflexilis*).

2.2. Photointerpretation and sampling

Our field and photointerpretation sampling protocol was based on a vegetation cover type map of an area over 42,000,000 ha developed by M.A. Kalkhan. The vegetation of the study site was stratified into 16 cover types based on 1:15,840 natural color aerial photographs acquired September 28,1987 (see Kalkhan et al., 2001). These cover types were delineated through photointerpretation using an Old Delft Scanning Stereoscope II and digitized using ARC/INFO GIS Software (Version 7.3; ESRI, 1999). We overlaid a grid north to south on the aerial photographs and randomly selected five to seven potential sample points in each of the 16 cover types using a random number generator. We located the points in the field with the aid of aerial photography, topographic maps, and a compass, and later checked and mapped the locations with a global positioning system (Trimble Pathfinder Professional GPS). All sampled field points located on aerial photography were linked to the remotely sensed imagery through their UTM coordinates based on double sampling design (see Kalkhan et al., 1995, 1998 for more details).

2.3. Pixel nested plot sampling design

During the summer field season of 2003, we established 60 pixel nested plots (PNP) based on stratified random sampling using existing vegetation cover type maps that encompassed our study area within RMNP (see Figs. 1 and 2). The PNP is a plot layout design of 225 m^2 (15 m × 15 m, Fig. 2). Fig. 2A is an example of remote sensing image pixels that the pixel nested plot is intended to represent.

The Landsat Enhanced Thematic Mapper plus (Landsat-7, ETM+) data consists of eight spectral wavelength bands with a nominal spatial resolution of 15 m (panchromatic mode), 30 m (6-multispectral modes), and two with 60 m (thermal modes). We used

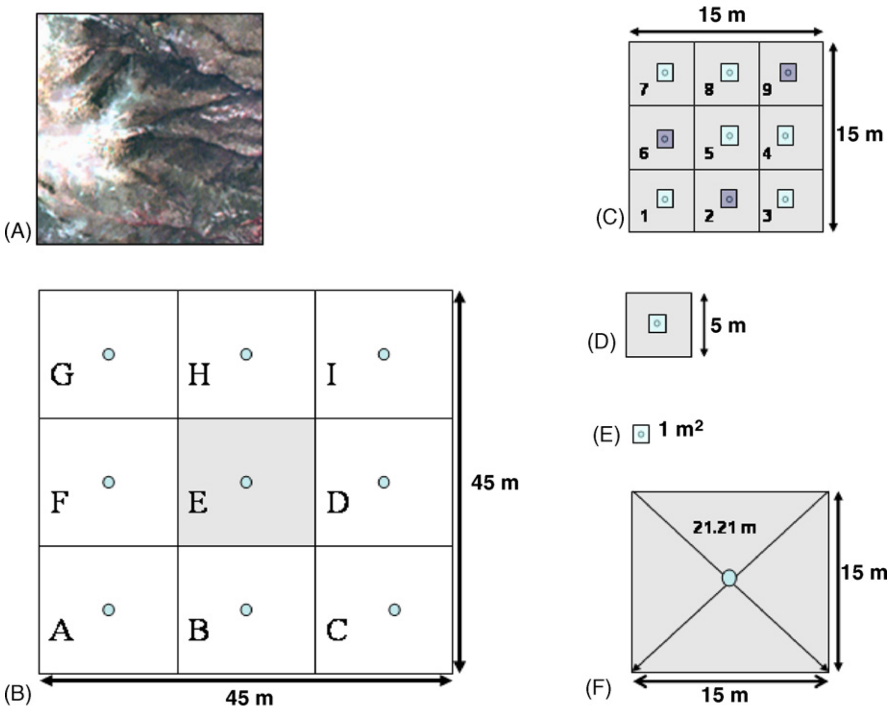

Fig. 2. Pixel diagram and layout for the pixel nested plot design used to collect vegetation, biotic and abiotic data within our study area, Rocky Mountain National Park, Colorado, USA.

a 15 m × 15 m layout for this rapid assessment sample of biotic and abiotic parameters because it eased the implementation of the field plots in the difficult mountainous terrain and because the plot represents the finest ground spatial resolution measured by the satellite. The multi-scale nested design of the PNP is also complimentary in terms of the ground spatial resolution to other satellite sensors such as IKONOS, Orbview 3, RADARSAT, and SPOT, which have even finer scale resolutions (ranging from 1 to 20 m^2). Satellite data provides unique information with full coverage of the landscape (each Landsat scene represents 185 km × 178 km = 32,930 km^2), which when coupled with field data can be a powerful tool for ecological and environmental assessments. Sampling was conducted within 60 randomly placed 225 m^2 plots of the study area (Figs. 1 and 2C represent the main 15 m × 15 m PNP), where the center points were established by first locating a random set of x and y UTM coordinates using a topographic map, aerial photography, and a GPS unit.

Each main 225 m^2 (15 m × 15 m) PNP consisted of nine nested 25 m^2 (5 m × 5 m, Fig. 2D) subplots and three 1 m^2 (1 m × 1 m, Fig. 2E) vegetation plots, with center points equal to the center points of three randomly selected 25 m^2 sub-plots. All plots and subplots were oriented to the north to ensure consistency in data sampling protocol over the landscape (Fig. 2C).

Next, three random numbers between one and nine were generated to designate the three 25 m^2 subplots to be used for field sampling (Fig. 2C). Intensive vegetation sampling (Fig. 2E) was completed within a 1 m × 1 m portable quadrat, placed around the center of the random subplot. Fig. 2F represents two crossing transects of 21. Twenty-one meter that can be used to collect biotic and abiotic data on riparian habitats, wetland environments, fuel loading, and burn severities. In addition, each 225 m^2 main pixel nested sample plot was surrounded by eight equally sized, 225 m^2 plots (15 m × 15 m) used for a thematic mapping field accuracy assessment (Fig. 2B).

2.4. Vegetation and soil (sampling and analysis)

Within each of the three random 1 m^2 vegetation subplots of PNP, we recorded cover percentage and average height (cm) of present vegetation by species. Cover percentages were recorded for eight abiotic variables (rock, soil, litter, standing duff, scat, wood, water, and duff). From each of the three vegetation plots an average depth for both duff and litter were recorded. Three representative soil samples were collected within the three random subplots (5 m × 5 m). Soil cores were 2 cm in diameter and taken at a depth of 15–20 cm and combined together to represent the 225 m^2 plot. These samples were air dried and sieved with a 2 mm sieve (number 10 standard sieve). Particle size was determined using the hydrometer method (Gee and Bauder, 1986). The samples were then ground using a roller mill. Percentage of total carbon (C) and nitrogen (N) concentrations were determined using a LECO-1000 CHN analyzer (Carter, 1993). Inorganic carbon was determined using a volumetric method described by Wagner et al. (1998), and analyzed using the pressure transducer (Sherrod et al., 2002). Organic carbon was determined by calculating the difference between total carbon and inorganic carbon (Kou, 1996). In addition, soil P was determined colorimetrically from a sodium bicarbonate extraction (see Kou, 1996). To determine exchangeable calcium (Ca), potassium (K), sodium (Na), and magnesium (Mg), the filtered extract of 25 ml of ammonium acetate and 5 g of soil buffered to pH 8.5 (see Summer and Miller, 1996; Bashkin et al., 2003) was analyzed by inductively coupled plasma emission spectrometry (Gee and Bauder, 1986).

Presence of vegetation by species and cover percentages for the abiotic variables were recorded for each of the three 25 m^2 sub-plots coinciding with the three randomly selected 1 m^2 plots. Counts and average heights (cm) by species of both tree seedlings and saplings were recorded for the three random 25 m^2 sub-plots. Canopy closure was estimated from the center of each of the random 25 m^2 sub-plots using a convex densiometer. Canopy closure estimates were used to calculate a measure of photosynthetically active radiation (PAR). PAR is defined as the amount of photosynthetically active (400–700 nm) light not intercepted by the forest canopy and available for understory vegetation (Gates, 1980; Kirkman et al., 2000). Ancillary data recorded for each plot included slope, aspect, x and y UTM location from the center of the plot and elevation from a global positioning system.

Each site was sampled as close to the vegetative phenological maximum (peak biomass) as possible. In Rocky Mountain National Park the time of peak biomass/phenological maximum is dependent upon the yearly precipitation, elevation gradient, and aspect orientation of each site, but for the entire study site this generally occurs between May and July. Approximately 10% of plant species could not be identified in the field and were collected but not included in the analysis. The unidentified plant species were identified at the Colorado State University herbarium and will be used in future analysis, as this study progresses. Species were classified as native or exotic according to the Natural Resource Conservation Service PLANTS database (USDA, 1999).

2.5. Data analysis

Canonical correspondence analysis is an integration of regression and ordination analyses into multivariate techniques for direct gradient analysis studies (ter Braak, 1986, 1987). This technique greatly improves the power to detect specific effects of cross variable association and has been shown to be a robust model for detecting the relationship between species and their environment (Palmer, 1993; Reed et al., 1993). This model is not constrained to linear relationships and can detect unimodal relationships between species (biodiversity) and environmental parameters (Bashkin et al., 2003). Cover of exotic plant species and soil nitrogen data were transformed using Log_{10} to approximate a normal distribution.

To characterize the relationships between our sampled plant species composition (cover and richness) and environmental measurements (e.g., soil characteristics, elevation), we used canonical correspondence analysis (CCA; PC-ORD Version 4.0; ter Braak, 1986, 1987). To test for the linear unimodal response of selected variables, a detrended correspondence analysis was performed (Palmer, 1993). Two separate datasets were used in CCA analysis. The first dataset contained percent cover for native and exotic plant species accompanied by the percent cover for nine overstory vegetation types (e.g., aspen cover). This dataset also included values for native and exotic plant species richness. The second dataset includes the plant and species richness and cover datasets which were evaluated against the same 10 environmental variables: organic carbon, sand, clay, nitrogen, sum of the cations (K, Na, Mg), elevation, aspect, slope, bare ground, and photosynthetically active radiation (PAR). We assessed all environmental variables for multicollinearity (high correlation between the independent variables) and none were found. Similar to ter Braak (1991), Monte Carlo permutation tests were performed to test the significance of the first axis using a randomization of the plots with 99 permutations. Finally, environmental variables used in CCA were used as independent variables in stepwise forward multiple regressions (SPSS, 2000) to predict native and exotic plant species cover and richness.

2.6. Spatial statistical analysis

Spatial autocorrelation statistics measure the level of interdependence between the variables and the nature and strength of the interdependence (Fortin et al., 1989). Spatial autocorrelation may be classified as either positive or negative (zero means no spatial pattern). Positive spatial autocorrelation has all similar values appearing together, while negative spatial autocorrelation has dissimilar values appearing in close association. Furthermore, spatial autocorrelation is related to the scale of the data as a periodicity of elements is assessed. Negative spatial autocorrelation is more sensitive to changes in scale. In ecological geographic applications there is usually positive spatial autocorrelation.

The most commonly used measures for spatial autocorrelation in ecological, health, environmental, and geological studies are the Moran's I statistic (Moran, 1948), Geary's C statistic (Geary, 1954), and the spatial cross-correlation statistic (Fortin et al., 1989; Bonham et al., 1995; Reich et al., 1995; Kalkhan and Stohlgren, 2000). These are especially useful in studies of patchily distributed resources in time and space (e.g., water, nutrients; Bartell and Brenkert, 1991) as determinants of vegetation patterns (Reich et al., 1995). Spatial autocorrelation and cross-correlation statistics can be used to evaluate spatially explicit information (e.g., information on the spatial pattern and scale between the variables of interest within landscape environments) on vegetation characteristics and structures (e.g., plant cover-abundance, patterns of old-growth forest stands, and plant species richness patterns), soil parameters, and associated environmental characteristics (e.g., topographic, edaphic, and resource availability; Reich et al., 1995; Kalkhan and Stohlgren, 2000).

These tests indicate the degree of spatial association as reflected in the data set as a whole. They both necessitate the choice of a spatial weights matrix. While Moran's I is based on cross products to measure value association, Geary's C employs squared differences (Legendre and Fortin, 1989). Moran's I behaves like a Pearson correlation coefficient. Its value is between -1 and $+1$ when scaled, otherwise it can exceed -1 or $+1$ (Legendre and Fortin, 1989). Geary's C is similar to a distance-type structure function and is within the range of 0 to +2 (Sokal and Oden, 1978). Values of C smaller than 1, correspond to a positive spatial autocorrelation, while values greater than 1 through 2 represent a negative spatial autocorrelation (Sokal and Oden, 1978). The greater the positive autocorrelation, the lower the value of C. Both Moran's I and Geary's C can be tested for significance against their theoretical distribution (Sokal and Oden, 1978; Cliff and Ord, 1981; Legendre and Fortin, 1989).

The cross-correlation statistic (I_{YZ}, Czaplewski and Reich, 1993) was used to test the null hypothesis of no spatial cross-correlation among all pairwise combinations of vegetation variables, topographic, and edaphic characteristics. Cliff and Ord (1981) showed that I_{YZ} ranges from −1 to +1, although it can exceed these limits with certain types of spatial matrices.

In calculating the various test statistics, inverse distance weighting was used to describe the spatial proximity of the sample plots to one another. All tests were preformed at the 0.05 level of significance. Significant cross-correlation does not imply a true cross-correlation. The test statistic is testing for both auto and cross-correlations simultaneously. If significant cross-correlations are observed additional testing may be required to ascertain the true nature of the relationship between variables. In this study, S-plus statistical software (Insightful Corporation, 2002) was used for spatial autocorrelation and cross correlations (Reich and Davis, 1998).

3. Results

3.1. Native and exotic species richness

The plots averaged 18.9 (±1.71) native plant species, and 0.9 (±0.2) exotic plant species (Table 1). Twenty-three of the 60 pixel nested plots contained at least one exotic plant species, while 15 contained two or more exotic plant species per 0.024 ha plot. The value 0.024 ha is equal to the 225 m^2 (15 m × 15 m) size of an individual pixel nested plot. On average native species cover was 46.4% (±3.8%), and exotic plant species cover was 1.0% (±0.3%) per plot (Table 1).

3.2. Species richness and cover by vegetation type

Plant species richness and cover varied among vegetation types (Table 1). Rocky alpine meadow plots averaged 23.5 (±6.5) native species, in comparison to spruce-fir, which averaged only 16.6 (±4.8). Meadows and ponderosa pine vegetation types also contained a relatively high degree of native species richness: 20.7 (±4.7) and 21.8 (±4.1), respectively (Table 1).

In general, exotic species plant richness and cover increased with native plant species richness and cover (Table 1; Fig. 3). The aspen types averaged 2.0 (±1.5), the riparian averaged 4.0 (±1.7), and the meadow 1.444 (±0.4) exotic species per 0.024 ha plot. In comparison, the vegetation types with dominant overstory trees of Douglas fir, lodgepole pine, and spruce-fir contained less than one exotic species per plot. The exception to this tend was the species-rich ponderosa pine type (21.8 ± 4.1 native species per plot), which averaged less than one exotic species per plot. Exotic plant species cover was highest in the aspen and riparian vegetation types: 4.9% (±4.6%) and 4.5% (±2.7%), respectively (Table 1).

3.3. Canonical correspondence analysis of native and exotic species richness and cover

Canonical correspondence analysis found that the centroids for native and exotic plant species are associated primarily with the increase of photosynthetically active radiation (PAR) and, to a lesser degree, the increased nitrogen in the soil (Fig. 4). Though similar, the centroid for native plant species cover was less strongly correlated with PAR in relation to the centroid for exotic plant species cover (Fig. 4). However, the centroids for exotic and native plant species richness are closely related and inversely correlated to the amount of bare ground present (Fig. 4).

The first two canonical axes explained 20.4% of the cumulative variation (or variability) for species richness and cover, while adding the third axis to the analysis can explain 25.6% of the variation (Fig. 4). Fig. 4 shows the

Table 1
Summary statistics for 9 vegetation types based on 60 pixel nested plots in Rocky Mountain National Park, Colorado, USA

Vegetation type	Number of plots	Number of native species	Number of exotic species	Native cover (%)	Exotic cover (%)
Ponderosa pine	16	21.8 ± 4.1	0.6 ± 0.2	49.9 ± 7.3	.02 ± .02
Lodgepole pine	12	11.6 ± 2.5	0.2 ± 0.2	41.9 ± 5.8	0.1 ± 0.1
Riparian/wet meadow	4	27.0 ± 7.2	4.0 ± 1.7	53.2 ± 15.9	4.5 ± 2.7
Rocky alpine meadow	2	23.5 ± 6.5	0.0	38.9 ± 12.6	0.0
Spruce-fir	5	16.6 ± 4.8	0.0	68.0 ± 19.4	0.0
Boulder field	2	17.0 ± 10.0	1.0 ± 1.0	28.3 ± 8.3	0.2 ± 0.2
Aspen	3	24.7 ± 10.4	2.0 ± 1.5	71.4 ± 34.1	4.9 ± 4.6
Douglas-fir	6	19.2 ± 3.0	0.8 ± 0.4	44.1 ± 13.1	0.1 ± .07
Dry meadow	9	20.7 ± 4.7	1.4 ± 0.4	35.4 ± 6.2	2.5 ± 0.9
All vegetation types	60	18.9 ± 1.7	0.9 ± 0.2	46.4 ± 3.8	1.0 ± 0.3

The values are given as mean with standard errors (S.E.).

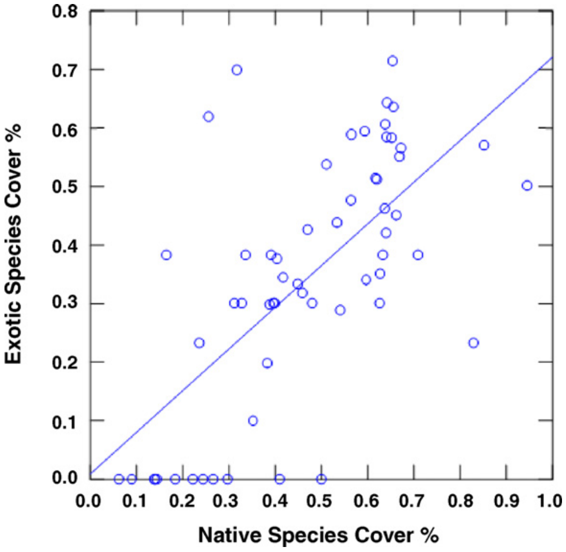

Fig. 3. Exotic plant species cover (%) vs. native plant species cover (%) within pixel nested plot study area, Rocky Mountain National Park, Colorado, USA.

top three abiotic factors that correlated significantly ($P < 0.05$) with canonical axis 1 and included: soil nitrogen (correlation coefficient $r = 0.58$), PAR ($r = 0.55$), and sum of cations ($r = -0.70$). The abiotic factor with the strongest correlation with canonical axis 2 was under-story bare ground ($P < 0.05$, $r = 0.66$). A sequential deletion technique used by Hix and Pearcy (1997) was implemented in this study to assess the influence of each environmental variable within our study on each canonical axis using Monte Carlo permutations. This procedure showed that the second canonical axis was significant (eigenvalue = 0.20; $P < 0.01$) (Table 2).

3.4. Predictors of native and exotic plant richness and cover

Multiple regression analysis showed that only a subset of the abiotic variables measured were significant predictors of native and exotic plant species richness and cover. Elevation and bare ground were the most common and significant of the variables measured. The combination of abiotic variables that explained 23.5% of the variation for exotic plant species cover was sand, slope, elevation, and PAR (Fig. 4). The percent of variation explained by abiotic factors alone was only 9.6–23.5% (Fig. 4).

Biotic variables (i.e., native and exotic plant species richness and number) accompanied by abiotic variables explained 40.7–50.3% of the variation in native and exotic plant species cover and richness (Fig. 4). By using backward stepwise regression with the number of

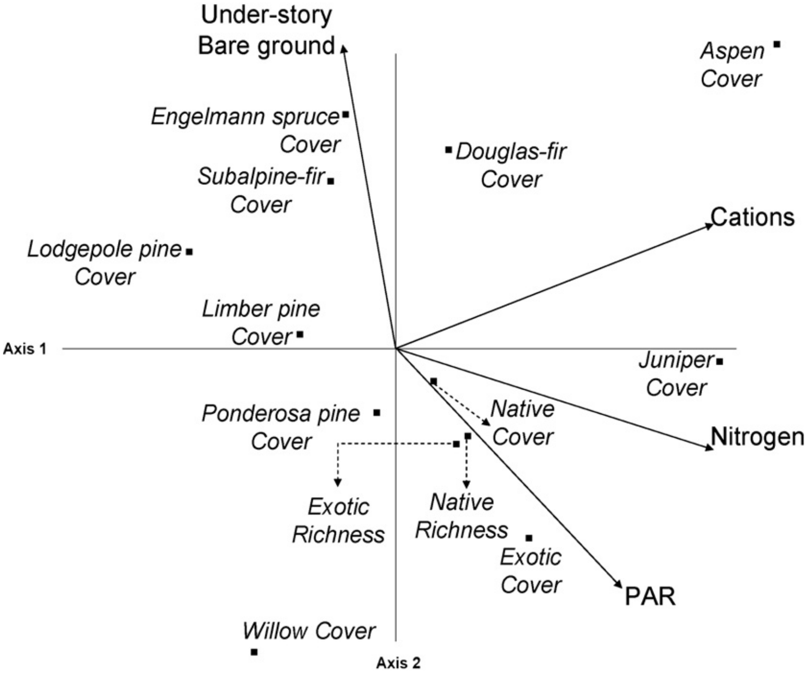

Fig. 4. Canonical correspondence analysis ordination for native and exotic plant species richness and cover within pixel nested plot study area, Rocky Mountain National Park, Colorado, USA.

Table 2
Stepwise multiple regressions for species richness and cover based on 60 pixel nested plots in Rocky Mountain National Park, Colorado, USA

Dependent variable/predictor	Coefficient	t	P	Model F, R^2, P
Native species richness				
Constant	8.003	3.561	0.001	$F = 4.013$, $R^2 = 0.096$, $P < 0.001$
Understory bare ground	−0.014	−1.748	0.086	
Elevation	−0.001	−2.162	0.035	
Exotic species richness				
Constant	1.986	3.328	0.002	$F = 10.783$, $R^2 = 0.407$, $P > 0.05$
Sum of cations	0.000	1.531	0.132	
Understory bare ground	−0.013	−2.687	0.010	
Clay	−0.030	−2.138	0.037	
Native species richness	0.317	4.044	0.000	
Exotic species cover				
Constant	10.081	2.498	0.016	$F = 3.424$, $R^2 = 0.078$, $P > 0.001$
Understory bare ground	−0.025	−1.792	0.079	
Elevation	−0.001	−1.838	0.071	
Number of exotic species				
Constant	0.306	2.888	0.006	$F = 20.245$, $R^2 = 0.503$, $P < 0.001$
Clay	−0.004	−1.589	0.118	
Understory bare ground	−0.002	−2.718	0.009	
Number of native species	0.625	5.953	0.000	
Exotic species cover				
Constant	0.803	1.817	0.075	$F = 5.367$, $R^2 = 0.235$, $P = 0.001$
Sand	0.006	2.237	0.029	
Slope	−0.005	−1.918	0.060	
Elevation	−0.000	−2.151	0.036	
Photosynthetically active radiation (PAR)	−0.004	−2.731	0.009	
Number of exotic species				
Constant	0.008	0.155	0.877	$F = 43.945$, $R^2 = 0.430$, $P < 0.001$
Number of native species	0.712	6.629	0.000	

exotic species as the dependent variable and the number of native species as the independent variable we explained 43% of the variation (Fig. 4).

3.5. Pearson's correlation, spatial auto-correlation, and cross correlation statistics

Bare ground was shown to have a linear negative correlation ($r = -0.23$; Table 3) with exotic plant species richness and their percent cover ($r = -0.23$). Native plant species richness had a positive linear correlation with exotic plant species richness ($r = 0.52$), while nitrogen content had a significant linear correlation with exotic plant species richness ($r = 0.21$) and native plant species percent cover ($r = 0.25$). PAR was negatively correlated ($r = -0.29$) to exotic plant species richness as well using the Pearson's correlation.

The sample plot locations were not uniformly distributed or equidistant from each other (Fig. 1). When we developed the spatial autocorrelation and cross-correlation statistics we used re-scaled UTM coordinates to develop a distance weight matrix (Reich and Davis, 1998). The inverse weight distance between sample plots was used as a weighting factor where weights were assigned by distance between plots. Plots with the closest proximity to one another were assigned greater weights than those farther apart.

In analyzing all 60 pixel nested plots sampled we found no variables to have significant spatial autocorrelation using the Moran's I and only one variable, nitrogen, was found to have significant autocorrelation using Geary's C statistic (Table 4). Based on all the plot locations these data would suggest that at the landscape level the number of native and exotic plant species were spatially independent throughout the study site, at least in relation to their own kind. It is possible however, that spatial patterns may occur at finer scales. Individual species may also exhibit spatial patterns across the landscape.

The cross correlation statistic was used to analyze spatial cross relationships between variables (Table 4). Native plant cover was found to have a negative

Table 3
Results of Pearson linear correlation based on 60 pixel nested plots in Rocky Mountain National Park, Colorado, USA

Variables	Native	Native cover	Exotic	Exotic cover	Nitrogen	OC[a]	PAR[b]	Bare ground
Native	1							
Native cover	0.46[c]	1						
Exotic	0.52[c]	0.13[d]	1					
Exotic cover	0.18[d]	0.08[d]	0.61[c]	1				
Nitrogen	0.132[d]	0.211[e]	0.247[f]	0.049[d]	1			
OC[a]	−0.1[d]	−0.26[f]	−0.19[e]	−0.20[e]	0.12[d]	1		
PAR[b]	−0.03[d]	0.28[f]	−0.29[d]	−0.18[d]	−0.31[f]	−0.06[d]	1	
Bare ground	−0.43[c]	−0.94[c]	−0.23[e]	−0.23[e]	−0.30[f]	0.21[e]	−0.17[d]	1

[a] OC defined as organic carbon.
[b] PAR defined as photosynthetically active radiation.
[c] Significant at $\alpha = 0.001$.
[d] Significant at $\alpha = 0.05$.
[e] Significant at $\alpha = 0.1$.
[f] Not significant (Zar, 1974).

spatial cross correlation with exotic plant species richness and organic carbon content and was positively spatially linked to PAR. Nitrogen had a negative spatial cross correlation statistic across the landscape with soil organic carbon content, which was expected, since they are both nutrient constituents of the soil. Organic carbon was also found to have a negative spatial cross correlation with PAR. This result is similar to the findings in Bonham et al. (1995), who suggested that temperate areas with dense canopy cover often have soils rich in organic carbon. Exotic plant species richness was found to be negatively spatially correlated with bare ground, reinforcing a recent hypothesis that areas of high species richness and high species cover are more susceptible to invasion (Stohlgren et al., 2003).

4. Discussion

4.1. Patterns of native and exotic plant species richness and cover

Exotic plant species can be found in nearly all vegetation types, but were found to be dominant in areas with less overstory cover and higher levels of nitrogen. This finding is consistent with previous studies of exotic plant species distribution by Stohlgren et al. (2001, 2003). The CCA showed that though both native and exotic plant species prefer these conditions, the centroids for exotic plant species richness and cover show a greater preference for them (Fig. 4). Though some specialized native plant species have taken advantage of certain areas (i.e., alpine, spruce-fir and

Table 4
Results of spatial auto-correlation using Moran's *I* and Geary's *C*, and cross correlation statistics using Bi-Moran's *I* based on 60 pixel nested plots in Rocky Mountain National Park, Colorado, USA

Variables	Native	Native cover	Exotic	Exotic cover	Nitrogen	OC[a]	PAR[b]	Bare ground
Native	−0.04[c], 1.03[c]							
Native cover	−0.02[c]	−0.02[c], 0.99[c]						
Exotic	−0.03[c]	−0.04[d]	−0.03[c], 1.02[c]					
Exotic cover	0.001[c]	−0.02[c]	−0.01[c]	−0.03[c], 1.04[c]				
Nitrogen	−0.001[c]	−0.01[c]	−0.02[c]	−0.001[c]	−0.02[c], 0.94[d]			
OC[a]	0.01[c]	−0.02[e]	0.02[c]	0.02[c]	−0.03[e]	0.01[c], 1.01[c]		
PAR[b]	−0.001[c]	0.02[e]	−0.01[c]	−0.004[c]	−0.01[c]	−0.03[d]	−0.02[c], 0.99[c]	
Bare ground	0.02[c]	0.03[c]	0.04[d]	0.03[c]	0.01[c]	0.02[c]	−0.02[c]	−0.04[c], 1.02[c]

Diagonal values are Moran's *I* and Geary's *C* Spatial Auto-correlations Statistics, respectively. Off diagonal values are cross correlation statistics (Bi-Moran's *I*).
[a] OC defined as organic carbon.
[b] PAR defined as photosynthetically active radiation.
[c] Significant at $\alpha = 0.05$.
[d] Significant at $\alpha = 0.1$.
[e] Not significant.

boulder fields), exotic plant species and their cover increase with native species and their cover throughout other vegetation types (Table 1; Fig. 3).

4.2. Role of abiotic variables in exotic plant invasions

Rocky Mountain National Park is very diverse in regard to forest-vegetation types and their associated topography. Available plant resources differ throughout these areas in regard to nutrients, light availability and moisture. The ability to predict exotic plant species on the landscape cannot be limited only to abiotic factors. CCA showed that the percent variance (variability) that can be explained by abiotic variables is 25.6%. CCA ordination (Fig. 4) showed that exotic and native plant species favor low light interception, high nitrogen and, to a lesser degree, other cations (i.e., Ca, Mg, K and Na). Multiple regression analysis showed a low response to predicting exotic or native plant species presence with abiotic factors alone, 10% and 8%, respectively. The areas with the most resources, such as nitrogen, light interception with ground vegetation and moisture, are the riparian, aspen, and meadow vegetation types and they illuminate this pattern of preferred establishment by plant species. This pattern of mutual establishment and success for native and exotic plant species suggests a focus for future studies. Studies that isolate single variables within these highly species rich vegetation types illustrate the best means for making a distinction between them. We believe that the inability of these abiotic variables to predict both native and exotic plant species on the landscape demonstrates a need to further isolate those variables found most crucial to their establishment and success.

4.3. Spatial and linear correlations

Useful information is provided for describing spatial relationships between native and exotic plant species richness with respect to soil characteristics when using spatial auto and cross correlation statistics (Kalkhan and Stohlgren, 2000). These spatial correlation methods are the building blocks and key inputs for predictive geospatial models and thematic maps (Kalkhan et al., 2001). Our 60 PNP were randomly located using a stratified sampling design. When we examined the spatial autocorrelations within each pixel nested plot the statistics suggested that native and exotic plant species are spatially independent across the area sampled in Rocky Mountain National Park. A statistically significant ($p < 0.001$) linear relationship was found between native and exotic plant species richness. Nitrogen was positively linked with exotic plant species richness while bare ground was negatively correlated with exotic plant species richness. These findings support previous research which has hypothesized that exotic plant species invasion may be more likely in areas of high native plant diversity and cover, and areas with nutrient rich soils (Kalkhan and Stohlgren, 2000; Stohlgren et al., 2003). Stohlgren et al. (2003) analyzed 2958 1 m^2 vegetation plots sampled in eight states and found highly significant positive relationships between native and non-native plant species richness. They noted that their ‘‘analysis raises serious doubts that high native plant species richness somehow decreases an area’s vulnerability to invasions by non-native plants’’ (Stohlgren et al., 2003).

4.4. Ecological and management implications

Nutrient rich soils appear more vulnerable to exotic plant species invasion than those with less-fertile soils. In addition, shifts in soil conditions brought about by air pollution (N deposition) or disturbance (fire, soil erosion, wildlife, and others) may shift the balance for native and exotic plant species locally (Bashkin et al., 2003). In concurrence with the Baskin et al., study (2003), our study also suggests that rare vegetation types appear more vulnerable than common vegetation types to invasion by exotic plant species. And in particular, aspen, meadow and riparian types, which are the most biologically diverse, are most likely to be heavily invaded. Thus, where soil fertility is high, disturbance may greatly enhance the invasion process (Stohlgren et al., 2003). This suggests that management may want to focus on the protection of relatively rare and biologically diverse habitat types which contain nutrient rich soils within the landscape of the park. Since this study found a strong correlation with soil (total) N, we would recommend an enhanced future study to see which N species will be the major determinate; organic N, NH_4, or NO_3, as these can vary greatly across vegetation types within landscape and soil characteristics.

The pixel nested plot design implemented in this study is a time and cost-efficient sampling method for researchers and managers to collect biotic and abiotic data. The small size and shape of the pixel nested plot make it a practical and easily adapted plot design for a multitude of landscape and research scenarios. The PNP can enable researchers to assess the potential for invasion by: (1) analyzing the relationships between abiotic and biotic variables; and (2) allowing for the efficient linkage of data to geospatial modeling and thematic mapping applications. This is due to the nature of topographic data derived from digital elevation models and remotely sensed data as input variables to geospatial models which are based on the square in shape, raster cell format. As a tool this will allow for the forecasting of ecological and environmental parameters at a specific time and location, such as patterns of plant diversity, species richness, areas vulnerable to invasion by exotic plant species, and fuel loading at landscape scales. Finally, for selection of sites in future research programs using different geospatial information data at large landscape scales, the pixel nested plot design is a potentially powerful tool. The PNP can be used with the intention to capitalize on remote sensing technology now readily available and inexpensive to acquire, while maintaining the benefits of a multi-scale nested plot design to characterize landscape patterns of plant diversity.

Acknowledgements

Partial funding for this research was provided by NASA and the Biological Resources Division of the United States Geological Survey. We thank Mr. Rick Shory for help with field work, plant identifications, and database analysis. Dr. Robin M. Reich, Dr. Deborah R. Spotts, and anonymous reviewers provided helpful editorial comments. Our thanks are to the staff of the Natural Resource Ecology Laboratory, Colorado State University, and Rocky Mountain National Park. To all we are grateful.

References

Bartell, S.M., Brenkert, A.L., 1991. A spatial-temporal model of nitrogen dynamics in a deciduous forest watershed. In: Turner, M.G., Gardner, R.H. (Eds.), Quantitative Methods in Landscape Ecology, Ecological Studies, vol. 82. Springer-Verlag, New York, pp. 379–398.

Bashkin, M., Stohlgren, T.J., Otsuki, Y., Lee, M., Evangelista, P.H., Belnap, J., 2003. Soil characteristics and plant exotic species invasion in the Grand Staircase-Escalante National Monument, Utah, USA. Appl. Soil Ecol. 22, 67–77.

Bonham, C.D., Reich, R.M., Leader, K.K., 1995. Spatial cross-correlation of *Boutelua gracilis* with site factor. Grassland Sci. 41, 196–201.

Brown, K.E., 2003. Mapping and modeling leafy spurge spread in Theodore Roosevelt National Park, North Dakota using spatial information and spatial statistics. Ph.D. Dissertation, Colorado State University, Fort Collins, CO, USA, p. 163.

Carter, M.R., 1993. Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, FL, USA.

Cliff, A.D., Ord, J.K., 1981. Spatial Processes, Models and Applications. Pion Ltd., London, England, pp. 21–45.

Czaplewski, R.L., Reich, R.M., 1993. Expected value and variance of Moran's bivariate spatial autocorrelation statistic under permutation. USDA Forest Service Research Paper, RM-309. Fort Collins, Colorado, pp. 1–13.

Daubenmire, R.F., 1959. A canopy-coverage method of vegetational analysis. Northwest Sci. 33, 43–64.

ESRI, 1999. Environment Systems Research Institute, Inc., 380 New York Street, Redlands, CA 92373, USA.

Fortin, M.J., Drapeau, P., Legendre, P., 1989. Spatial auto-correlation and sampling design in plant ecology. Vegetatio 83, 209–222.

Gates, D.M., 1980. Biophysical Ecology. Springer-Verlag, New York, 611 pp.

Geary, R.C., 1954. The contiguity ratio and statistical mapping. Incorp. Stat. 5, 115–145.

Gee, G.W., Bauder, J., 1986. Particle size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1—Physical and Mineralogical Methods. ASA, Madison, Wisconsin, pp. 383–411.

Harper, K.T., Van Buren, R., Kitchen, S.G., 1996. Invasion of alien annuals and ecological consequences in salt desert shrublands of western Utah. In: Barrow, J.R., McArthur, E.D., Sosebee, R.E., Tausch, R.J. (Eds.), Shrubland Ecosystem Dynamics in a Changing Environment. Technical Report INT-GTR-338, Ogden, UT, pp. 58–65.

Hix, D.M., Pearcy, J.N., 1997. Forest ecosystems of the Marietta Unit, Wayne National Forest, southeastern Ohio: multifactor classification and analysis. Can. J. For. Res. 27, 1117–1131.

Insightful Corporation, 2002. S-plus, Seattle, WA 98109, USA.

Joy, S.M., Reich, R.M., Reynolds, R.T., 2003. A non-parametric supervised classification of vegetation types on the Kaibab National Forest using decision trees. Int. J. Rem. Sen. 9, 1836–1852.

Kalkhan, M.A., Stohlgren, T.J., Coughenour, M.B., 1995. An investigation of biodiversity and landscape-scale gap patterns using double sampling: A GIS approach. In: Proceeding of the Ninth Conference on Geographic Information Systems, Vancouver, British Columbia, Canada, pp. 708–712.

Kalkhan, M.A., Reich, R.M., Stohlgren, T.J., 1998. Assessing the accuracy of Landsat Thematic Mapper classification using double sampling. Int. J. Rem. Sen. 19, 2049–2060.

Kalkhan, M.A., Stohlgren, T.J., 2000. Using multi-scale sampling and spatial cross-correlation to investigate patterns of plant species richness. Environ. Monit. Assess. 64, 591–605.

Kalkhan, M.A., Stohlgren, T.J., Chong, G.W., Schell, L.D., Reich, R.M., 2001. A predictive spatial model of plant diversity: integration of remotely sensed data, GIS, and spatial statistics. In: Proceeding of the Eight Forest Remote Sensing Application Conference (RS 2000). April 10–14, 2000, Albuquerque, New Mexico, (CD-ROMs Publications (ISBN 1-57083-062-2), p. 11.

Kalkhan, M.A., 2005. A new approach for landscape-scale assessment: Integration of geospatial information-statistics with pixel plot sampling design. In: Aguirre-Bravo, Celedonio, (Eds.), Monitoring Science and Technology Symposium: Unifying Knowledge for Sustainability in the Western Hemisphere. September 20–24, 2004, Denver, CO. Proceedings RMRS-P-37-CD. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Ogden, UT.

Kirkman, L.K., Goebel, P.C., West, L., Drew, M.B., Palik, B.J., 2000. Depressional wetland vegetation type: a question of plant community development. Wetland 20, 373–385.

Kou, S., 1996. Phosphorus. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science Society of America, Madison, WI, pp. 869–920.

Legendre, P., Fortin, M.J., 1989. Spatial analysis and ecological modelling. Vegetatio 80, 107–138.

Metzger, K., 1997. Modeling small-scale spatial variability in stand structure using remote sensing and field data. M.S. Thesis, Department of Forest Sciences, Colorado State University, Fort Collins, CO 80523.

Moran, P.A.P., 1948. The interpretation of statistical maps. R. Stat. Soc. Ser. B 10, 243–351.

Palmer, M.W., 1993. Putting things in an even better order: the advantages of canonical correspondence analysis. Ecology 74, 2215–2230.

Parker, K.W., 1951. A Method for Measuring Trend in Range Condition on National Forest Ranges. .

Reed, R.A., Peet, R.K., Palmer, M.W., White, P.S., 1993. Scale dependence of vegetation–environment correlations: a case study of a North Carolina piedmont woodland. J. Veg. Sci. 4, 329–340.

Reich, R.M., Czaplewski, R.L., Bechtold, W.A., 1995. Spatial cross-correlation of undisturbed natural shortleaf pine stands in northern Georgia. Environ. Ecol. Stat. 1, 201–217.

Reich, R.M., Davis, R.A., 1998. On-line Spatial Library for the S-Plus Statistical Software Package. Colorado State University, Fort Collins, CO.

Reich, R.M., Lundquist, J.E., Bravo, V.A., 2004. Spatial Model for estimating fuel loads in the Black Hills, South Dakota, USA. Int. J. Wildland Fir. 13, 119–129.

Sherrod, L.A., Dunn, G., Peterson, G.A., Kolberg, R.L., 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Sci. Soc. Am. J. 66, 299–305.

Short, S.K., 1987. 27 Years of exotic plant control in Rocky Mountain National Park—summary and recommendations, RMNP Resource Management Report No. 1, p. 1.

Smith, S.D., Bunting, S.C., Hironaka, M., 1986. Sensitivity of frequency plots for detecting vegetation change. Northwest Sci. 60, 279–286.

Smith, S.D., Bunting, S.C., Hironaka, M., 1987. Evaluation of the improvement in sensitivity of nested frequency plots to vegetational change by summation. Great Basin Nat. 47, 299–307.

Sokal, R.R., Oden, N.L., 1978. Spatial autocorrelation in biology: 1. Methodology. Biol. J. Linnean Soc. 10, 199–228.

SPSS, 2000. SYSTAT, Version 9.0. Systat Software, Inc., 1737 Technology Drive, Suite 430, San Jose, CA 95110.

Stohlgren, T.J., Falkner, M.B., Schell, L.D., 1995. A modified-Whittaker nested vegetation sampling method. Vegetatio 117, 113–121.

Stohlgren, T.J., Binkley, D., Chong, G.W., Kalkhan, M.A., Schell, L.D., Bull, K.A., Otsuki, Y., Newman, G., Bashkin, M., Son, Y., 1999. Exotic plant species invade hot spot of native plant diversity. Ecol. Monogr. 69, 25–46.

Stohlgren, T.J., Otsuki, Y., Villa, C., Lee, M., Belnap, J., 2001. Patterns of plant invasions: a case example in native species hotspots and rare habitats. Biol. Inv. 3, 37–50.

Stohlgren, T.J., Barnett, D., Kartesz, J., 2003. The rich get richer: patterns of plant invasions in the United States. Front. Ecol. Environ. 1, 11–14.

ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.

ter Braak, C.J.F., 1987. The analysis of vegetation–environment relationships by canonical correspondence analysis. Vegetatio 69, 69–77.

ter Braak, C.J.F. 1991. CANOCO, Version 3.12.

Townsend, P.A., Walsh, S.J., 2001. Remote sensing of forested wetlands: application of multitemporal and multispectral satellite imagery to determine plant community composition and structure in southeastern USA. Plant Ecol. 157, 129–149.

USDA, 1999. The PLANTS Database. National Plant Data Center, Baton Rouge, LA, 70870. http://plants.gov/plants.

USDA Forest Service, http://www.fia.fs.fed.us/tools-data/.

USGS-NPS Vegetation Mapping Program, http://biology.usgs.gov/npsveg/standards.html.

Wagner, S.W., Hanson, J.D., Olness, A., Voorhees, W.B., 1998. A volumetric inorganic carbon analysis system. Soil Sci. Am. 62, 690–693.

Woodall, C.W., Graham, J.M., 2004. A technique for conducting point pattem analysis of clusterplot stem maps. For. Ecol. Manag. 198, 31–37.

Woldendorp, G., Keenan, R.J., Barry, S., Spencer, R.D., 2004. Analysis of sampling methods for coarse woody debris. For. Ecol. Manag. 198, 133–148.

Zar, J.H., 1974. Biostatistical Analysis. Prentice-Hall, INC, Englewood Cliffs, NJ.