

The intersection of ecological risk assessment and plant communities: an analysis of *Agrostis* and *Panicum* species in the northeastern U.S.

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Abstract Ecological risk assessments for grass species with novel traits are advisable, or required, in order to identify potential environmental harms prior to large-scale cultivation. Credible risk assessments are built upon knowledge of the communities that could be negatively affected by crop-to-wild gene flow, new weeds, or invasive plants. This study focused on two cultivated grasses with different life histories: the exotic, weedy *Agrostis stolonifera* (creeping bentgrass) and the native *Panicum virgatum* (switchgrass). Vascular plant communities were analyzed in 190 transects (50 m) in ten habitat types across two ecoregions (inland and coastal) in the northeastern U.S. Ordination plots and dendrogram analysis showed clustering of inland plant community assemblages within habitat types, while coastal plant communities were similar across the habitats studied. *Agrostis* and *Panicum* species had unequal distribution across the habitat types and ecoregions. *Agrostis* species were more common in the inland ecoregion and habitats receiving moderate management or disturbance events. In both ecoregions, *A. stolonifera* had high co-occurrence values with other exotic *Agrostis* species, suggesting potential for interspecific gene flow. *P. virgatum* was most common in inland

roadside and wasteland habitats, but was distributed equally in the three coastal habitats. Co-occurrence between *P. virgatum* and congenics was infrequent, although one transect had both *P. virgatum* and the state-listed coastal species *Panicum amarum*. This is the first study to characterize *Agrostis* and *Panicum* plant communities and distribution providing the basis for ecological risk assessments, coexistence-strategies, and geographic exclusion zones.

Keywords Habitat · Plant community · *Agrostis* · Creeping bentgrass · *Panicum* · Switchgrass · Ecological risk assessment

Abbreviations

- GE Genetically-engineered
CHP Lower New England Ecoregion subecoregion
Southern New England Coastal Hills and Plains
CL North Atlantic Coast Ecoregion, subecoregion
Southern New England Coastal Lowland
ERA Ecological risk assessment

Introduction

Grasses provide much of the world's food, but they also act as weeds and invasive species in diverse agricultural, natural, and cultural settings. In the U.S., a national energy initiative has identified a number of

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C₄ grasses as potential biofuel feedstocks, but some of these species are considered weedy or invasive plants (Raghu et al. 2006). Thus, the large-scale production of these plants could lead to management or ecological problems. The development of genetically engineered (GE) grasses has raised concerns about the downstream harm from crop-to-wild or crop-to-weed gene flow, especially if the novel phenotype increases stress resistance, fecundity, or overall fitness (Andow and Zwahlen 2006; Stewart et al. 2003; Wilkinson and Tepfer 2009). In addition, preventing gene flow in wind-pollinated grasses poses a challenge, and the containment problem increases when sexually compatible species (receiving populations) exist in natural, agricultural, or cultural landscapes (defined as properties representing the combined work of nature and man UNESCO 2010). The escape of glyphosate-resistant *Agrostis stolonifera* (creeping bentgrass) in the western U.S. provides an example of the challenges involved in biocontainment (Reichman et al. 2006; Watrud et al. 2004; Zapiola et al. 2008).

The harmonization of policy for GE crops across many countries has resulted in a regulatory process that requires the construction of predictive ecological risk assessments (ERA) prior to commercialization (Andow and Zwahlen 2006; Auer 2008; Craig et al. 2008; Nap et al. 2003). Risk analysts and regulators generally assess GE crops using a case-by-case approach with detailed knowledge about the biology of the crop, the biotechnology-derived trait, and the environment in which the crop will be grown (EPA 1998). The biggest challenges today in ERA are the identification of potential harms to valued communities and ecosystems (problem formulation), and research that allows quantification of future ecological changes (Wilkinson and Tepfer 2009). Concerns about the impact of novel grasses are not theoretical; non-native grass invasion has been associated with the decreased abundance of birds and arthropods in comparison to native grassland (Flanders et al. 2006).

In the case of ERA for cultivated grasses, understanding plant community dynamics is important to characterize direct or indirect harms to non-target species and the distribution of receiving populations for crop-to-wild gene flow. Unfortunately, regional floras and botanical references are generally insufficient to assess these risks because they are often

incomplete, out-of-date, or they do not include information about cultural or agricultural landscapes (Barkworth et al. 2007; Magee and Ahles 2007; Gleason and Cronquist 1963). In addition, botanical references typically have no information about the co-occurrence of closely related species that could favor interspecific gene flow. This information is critical for the design of geographic exclusion zones, regions where specific GE crops are not permitted because of potential for crop-to-wild gene flow (McGinnis et al. 2010). Our project was designed to provide information about the distribution, habitat types, and plant communities for two grass species that have been modified through traditional breeding and biotechnology to create novel traits for the U.S. market: *A. stolonifera* (creeping bentgrass) and *Panicum virgatum* (switchgrass).

Agrostis stolonifera is a cool-season, perennial grass planted in golf courses and other cultural landscapes in the U.S. (Beard 2002). Most references treat *A. stolonifera* as an introduced species in the U.S., but some sources cite specific ecotypes (or populations) as native (Barkworth et al. 2007). Experimental field trial permits have been issued for GE *A. stolonifera* (2002–2008) tolerant to herbicides, insects, drought, salt, heat, disease, and shade (USDA 2010). Male sterility and altered plant morphology have also been tested in experimental field trials. Herbicide-resistant *A. stolonifera* has generated the most research and scientific debate regarding gene flow and long-term ecological impacts, and it has escaped experimental field trials in the western U.S. (Reichman et al. 2006; Watrud et al. 2004; Zapiola et al. 2008). *A. stolonifera* and some closely related species are perennial weeds in the U.S. and other countries (Behrendt and Hanf 1979; MacBryde 2006). They are also considered to be invasive in some natural areas (Invasive Plant Atlas 2009; Levine 2000). Bentgrasses can be dispersed by seed and vegetative stolons, the latter being a trait common to many weedy and invasive plants (Oftinowski and Kenkel 2008; Cadotte et al. 2006). Intraspecific and interspecific pollen-mediated gene flow has been documented for *Agrostis* in experimental and natural conditions (reviewed in MacBryde 2006). *A. stolonifera* and the closely related *A. gigantea* are able to hybridize and form persistent populations in roadsides (Hart et al. 2009). However, there is little information about their habitat types, plant community

associations, or species co-occurrence in today's natural and managed ecosystems.

Panicum virgatum is a native, warm-season, perennial grass most commonly associated with the tall grass prairies of North America (Weaver and Fitzpatrick 1934). However, in northeastern North America, the presettlement distribution pattern for switchgrass is believed to have been a narrow zone adjacent to the coastal salt marsh (Niering and Warren 1980). Switchgrass is commonly sub-divided into two broad ecotypes, upland and lowland. Lowland ecotypes are notable for their larger stature and are typically tetraploids, while Upland ecotypes are somewhat smaller and can be either tetraploid or octaploid (Martinez-Reyna and Vogel 2002; Porter 1966). Switchgrass is an outcrossing species with strong self-incompatibility, increasing the likelihood of intraspecific gene flow (Martinez-Reyna and Vogel 2002). In the northeastern U.S., the *Panicum* genus includes native species growing in natural areas, native species established as weedy populations, and one non-native species that can be a crop or weed (*P. miliaceum*, Proso millet). As a cultivated plant, switchgrass 'source identified' cultivars have been used for livestock forage, wildlife habitat, prairie restoration, horticulture, and erosion control. In recent years, switchgrass has been promoted as a potential lignocellulosic feedstock for ethanol production (Wright and Turhollow 2010). Experimental field trial permits have been issued for GE *P. virgatum* (2008–2010) with increased biomass, decreased lignin, improved digestibility, improved nitrogen use efficiency, increased drought tolerance, or sterility (USDA 2010).

Our study was designed to address the following ecological questions in natural, cultural and agricultural landscapes: (1) What is the current distribution of *Agrostis* and *Panicum* species with respect to inland and coastal ecoregions, habitat types, and plant community assemblages in the northeastern U.S.?, (2) Do species co-occur in plant communities such that interspecific pollen-mediated gene flow could be promoted?, and (3) Are management or disturbance events commonly associated with *Agrostis* and *Panicum* populations? In addition to increasing knowledge about plant ecology, answers to these questions could inform decisions about field testing and commercialization of novel *Agrostis* and *Panicum* genotypes and traits.

Materials and methods

Study site, botanical surveys, and habitat definitions

Field studies were conducted in 2009 and 2010 in two ecoregions: Lower New England Ecoregion (subecoregion Southern New England Coastal Hills and Plains, abbreviated CHP) and North Atlantic Coast Ecoregion (subecoregion Southern New England Coastal Lowland, abbreviated CL) (Metzler and Barrett 2006; TNC 2010) (Fig. 1). The CHP transects were located within the western boundary of $72^{\circ}32'15.1218''$ to the eastern boundary of $71^{\circ}48'8.1678''$ and the northern boundary of $42^{\circ}1'18.9948''$ to the southern boundary of $41^{\circ}25'33.927''$. The CL transects along the Long Island Sound were located within the western boundary of $73^{\circ}30'2.6202''$ to the eastern boundary of $71^{\circ}27'24.3642''$ W and from the northern boundary of $41^{\circ}26'3.9186''$ to the southern boundary of $41^{\circ}02'37.0644''$.

Seven habitats were defined in the CHP subecoregion: core forest, edge forest, herbaceous meadow, roadside, wasteland, hayfield, and cornfield. Core forest transects were used as a comparator because they are the closest available representation of undisturbed, climax vegetation in this region. Core forest was defined by a forest fragmentation map based on three criteria: (1) second growth forest, (2) at least 91.44 meters to any non-forested area in all directions, and (3) total forest area of 202.34 hectares or greater (Fig. 1) (CLEAR 2006). Twenty core forest transects were distributed across the CHP region, transects representing the other six habitat types were clustered as close as possible to the core forest using the criteria listed below and the presence of the species of interest. Edge forest was defined as the transition between second growth forest and herbaceous plant communities and transects were laid perpendicular to the transition zone. Herbaceous meadows were defined based on the dominance of herbaceous plants and maintenance through mowing or suppression of woody plants (Metzler and Barrett 2006). Roadside transects were defined as locations within 2 m along the margins of two-lane state highways. Wasteland transects were defined as areas with extensive, recent human management activities and/or disturbance (e.g., gravel parking lots and the edges of dirt roads). Hayfields were defined by the

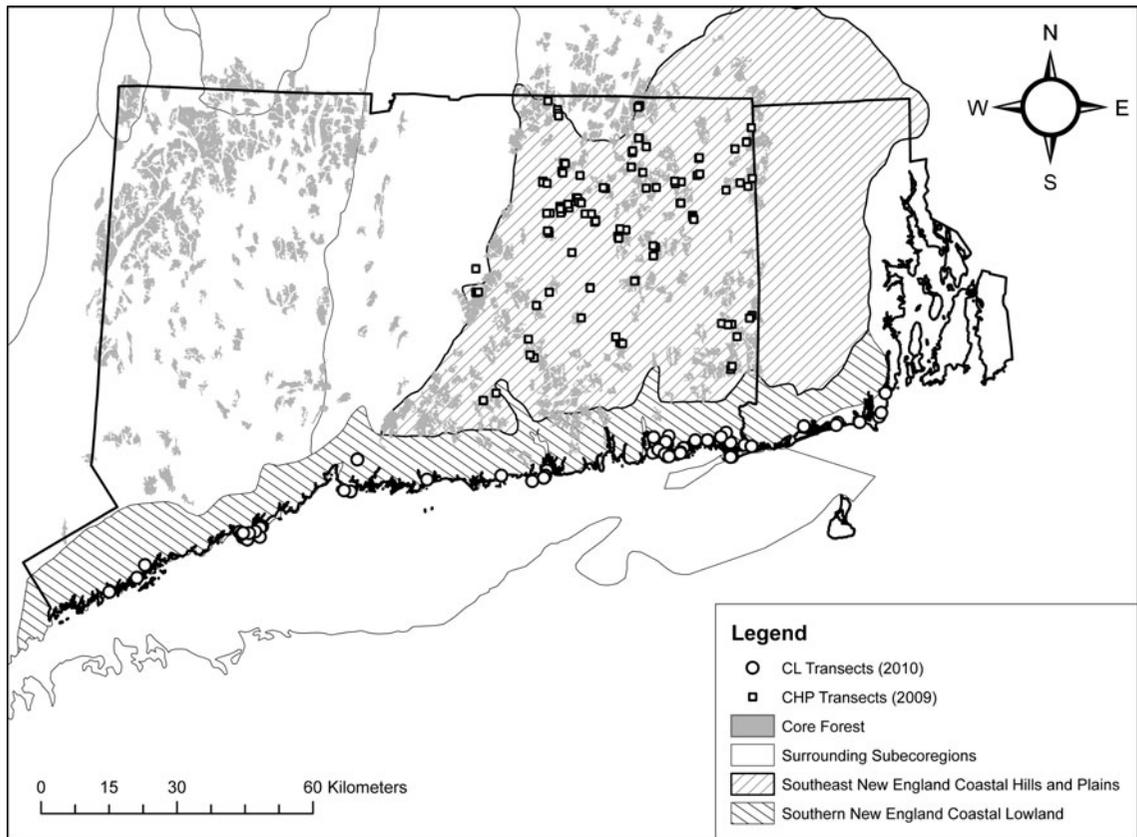


Fig. 1 Location of transects (190) in ten habitat types in two ecoregions in the northeastern United States: Lower New England Ecoregion (*CHP* subcoregion Southern New England Coastal Hills and Plains), and North Atlantic Coast Ecoregion

presence of crop species (e.g., orchard grass, smooth brome, timothy, and clovers) with mowing and harvesting 2–3 times per year. Cornfield edges (cornfield) were defined by the presence of *Zea mays* grown as an agronomic row crop. Transects ran along the edge of the cultivated field and included the last row of corn plants. Throughout the study, disturbance has been defined as any discrete event that disrupts the ecosystem, community, or population structure and changed resources, substrate availability, or the physical environment (White and Pickett 1985).

Transects representing three habitat types in the coastal CL subcoregion were difficult to establish because most of the coastal land is privately owned and disturbed. Transects were replicated in 20 locations for CL semi-natural, human impacted, and roadside (Fig. 1). The CL semi-natural habitat was defined as a coastal area with infrequent human

CL subcoregion Southern New England Coastal Lowland, abbreviation). Inland ecoregion transects were clustered near core forest habitat. Transects were conducted in two consecutive years

traffic and where plant communities experienced minimal management. The CL human impacted habitat was defined as a coastal area with frequent human traffic and often managed as part of a recreation area (e.g. beach, boat launch). The CL roadside habitat was defined by the same criteria as the CHP roadside habitat.

Plant communities can be defined as the collection of species at a specified place and time (Magurran 2004); in our study, we defined a plant community as the taxa observed along a transect. Transect surveys (50 m) were conducted in late summer (July–September) and were replicated at twenty locations in each habitat type except for hayfields which were replicated 10 times due to low plant species diversity. The beginning and end points of each transect was recorded using a Juno SB handheld GPS (Trimble Navigation Limited, Westminster, CO USA). Tree canopy (shade)

was measured along each transect at 10, 25, and 40 m using a spherical densiometer (Model-C, Forest Densiometers, Bartlesville, OK) and then averaged. A 50-m measuring tape was laid on the ground and every vascular plant that fell directly above or below was identified and the number of ramets counted; this data was used for all calculations. In addition, target species (*Agrostis*, *Panicum*, and invasive plants) were recorded in a belt extending 1 m on each side of the transect line. Belt transects provided information about presence/absence, but not community composition or co-occurrence values. *Carex* and *Juncus* species were classified to the genus level. Plant species nomenclature and nativity were as defined by the National PLANTS Database (2010). Invasive plants for the region were based upon a list from the Connecticut Invasive Plant Council (2009).

Data analysis

Records of the vascular plants and environmental variables observed in 190 transects were entered into Excel for data management (Microsoft Corporation, Redmond, WA). Species diversity was calculated using Shannon's diversity index (SDI) because there were many singletons and SDI is sensitive to rare occurrences (Gurevitch et al. 2006). Two-way analysis of variance (transects were random effects, habitats were fixed effects) was used to determine differences between SDI values (SAS ver. 9). Nonmetric multidimensional scaling (NMDS) was used to compare dissimilarities among plant communities (Kruskal 1964) and was computed using the Bray–Curtis method (Bray and Curtis 1957) in R with the vegan package (R Development Core Team 2009, Oksanen et al. 2009) and plotted using Sigmplot v10 (Systat, Chicago, IL). The statistical package R computed relationships between communities by calculating the closest representation on a two axis plot. The r^2 value was determined by calculating the correlation between actual dissimilarity and plotted dissimilarity (how well the plot configuration matches the data). Using the dissimilarities, we plotted the habitats in a hierarchical cluster analysis dendrogram using the single linkage method in the vegan package in R (Oksanen 2009). Branch length equaled "height", the measure of similarity of the joined clusters.

Co-occurrence was computed for *A. stolonifera* and *P. virgatum* using the formula:

$$C_{ih} = 1 - 0.5 \sum | p_{ij} - p_{hj} |$$

(Schoener 1969, Silva and Batalha 2009). In this equation, C is the co-occurrence value of the i th and h th species, and p is the proportion of occurrence of the i th or h th species in the j th community (transect). Our project compared habitats where plants differed in size. In order to compensate for size differences, we calculated the co-occurrence data using presence/absence information from transects instead of species abundance values.

Results

Vascular plants and habitat types

In the inland CHP region, 366 vascular plant species and 53,720 individuals were recorded in 130 transects conducted across seven habitat types (Table 1). The most abundant plant species across all transects was *Digitaria sanguinalis* (5,144 ramets) which was also the most common species (presence/absence) in CHP roadside habitats (Table 1). There were twenty singletons in 130 CHP transects. The seven CHP habitats were distinguished from each other by their plant communities and one environmental factor (canopy cover) (Table 1). Core forests had the second lowest values for SDI (7.08); only cultivated hayfields had less plant species diversity. Core forests had very few exotic or invasive plants and the most common species were native, consistent with our belief that these transects were the closest representation of undisturbed native vegetation (Table 1). Core forest transects had the most shade (86% canopy cover) and significantly more canopy cover than edge forest (65%) or other habitat types. Edge forest habitat had the highest SDI (Table 1). Even though the % invasive plant ramets was relatively high, the value for % exotic (non-native) ramets was not significantly different compared to meadow, wasteland, or roadsides (Table 1). Similar to core forests, the five most common species were native plants. Plant diversity was relatively high for meadows, wastelands, and roadsides but not significantly different from one another; the exotic species *A. stolonifera* was among the five most common species in these habitats. The habitats with the lowest plant diversity indices were cornfields and hayfields. The high % of exotic plants in these two habitats was

Table 1 Habitat types studied in the Lower New England ecoregion, Southern New England Coastal Hills and Plains (CHP) subecoregion, and the North Atlantic Coast ecoregion, Southern New England Coastal Lowlands (CL) sub-ecoregion

Habitat	n_t	$e^{H'}$	%CC	%inv	% exotic	# species	# spp/ transect	Five most common species
CHP Core Forest	20	7.08 ^{de}	86 ^a	2 ^b	2 ^d	71	3.55 ^{de}	<i>Acer rubrum</i> , <i>Thelypteris noveboracensis</i> , <i>Quercus rubra</i> , <i>Vaccinium corymbosum</i> , <i>Hamamelis virginiana</i>
CHP Edge Forest	20	13.80 ^a	65 ^b	15 ^a	28 ^c	187	9.35 ^a	<i>Acer rubrum</i> , <i>Thelypteris noveboracensis</i> , <i>Solidago rugosa</i> , <i>Vaccinium corymbosum</i> , <i>Parthenocissus quinquefolia</i> , <i>Vaccinium corymbosum</i> , <i>Parthenocissus quinquefolia</i>
CHP Meadow	20	11.20 ^b	7 ^c	6 ^b	34 ^c	181	9.05 ^{ab}	<i>Rubus occidentalis</i> , <i>Solidago rugosa</i> , <i>Solidago graminifolia</i> , <i>Agrostis stolonifera</i> , <i>Potentilla simplex</i>
CHP Wasteland	20	10.47 ^{bc}	14 ^c	10 ^a	35 ^c	177	8.85 ^b	<i>Dichanthelium clandestinum</i> , <i>Solidago rugosa</i> , <i>Conyza canadensis</i> , <i>Agrostis stolonifera</i> , <i>Solidago graminifolia</i>
CHP Roadside	20	8.25 ^{cd}	15 ^c	4 ^b	32 ^c	138	6.90 ^c	<i>Digitaria sanguinalis</i> , <i>Dichanthelium clandestinum</i> , <i>Ambrosia artemisiifolia</i> , <i>Chaenorhinum minus</i> , <i>Agrostis stolonifera</i>
CHP Cornfield	20	5.25 ^{ef}	14 ^c	1 ^b	64 ^b	113	5.65 ^{cd}	<i>Zea mays</i> , <i>Digitaria sanguinalis</i> , <i>Ambrosia artemisiifolia</i> , <i>Panicum dichotomiflorum</i> , <i>Setaria faberi</i>
CHP Hayfield	10	4.39 ^f	0 ^c	8 ^{ab}	96 ^a	57	5.70 ^c	<i>Dactylis glomerata</i> , <i>Phleum pratense</i> , <i>Trifolium purpurea</i> , <i>Plantago lanceolata</i> , <i>Galium mollugo</i>
CL Human Impacted	20	6.59 ^a	3 ^a	15 ^a	30 ^a	127	6.4 ^a	<i>Solidago sempervirens</i> , <i>Panicum virgatum</i> , <i>Phragmites australis</i> , <i>Oenothera biennis</i> , <i>Celastrus orbiculatus</i> , <i>Ambrosia artemisiifolia</i>
CL Semi-Natural	20	4.90 ^a	0 ^a	12 ^a	21 ^a	118	5.9 ^a	<i>Panicum virgatum</i> , <i>Solidago sempervirens</i> , <i>Celastrus orbiculatus</i> , <i>Toxicodendron radicans</i> , <i>Solidago graminifolia</i>
CL Roadside	20	8.74 ^a	7 ^a	5 ^a	28 ^a	124	6.2 ^a	<i>Panicum virgatum</i> , <i>Digitaria sanguinalis</i> , <i>Celastrus orbiculatus</i> , <i>Ambrosia artemisiifolia</i> , <i>Dichanthelium clandestinum</i>

Letters indicate significant differences between habitats within each ecoregion ($\alpha = 0.05$). n_t , number of transects observed; $e^{H'}$, mean Shannon's diversity index; % CC, mean % transect covered by tree or shrub canopy; %inv, mean % invasive plant species (# invasive ramets/# ramets in transect); %exotic, mean % exotic plant species (# non-native ramets/# ramets in transect); # species, number plant species observed in habitat type; #species/transect, mean number of plant species per transect; five most common species, five most common species based on presence/absence in transects

consistent with the cultivation of crop species and weed management practices. The most common species included crops (e.g. *Zea mays*, *Trifolium purpurea*) and agricultural weeds (e.g. *Panicum dichotomiflorum*, *Digitaria*, *Plantago*). The relatively high % of invasive ramets in hayfields was due to one transect with many *Euphorbia cyparissias* ramets.

Transects in the CL region (60) near Long Island Sound contained 196 vascular plant species, 16,925 individuals, and 15 singletons. The most abundant species were *P. virgatum* (3,544 ramets) and *D. sanguinalis* (1,132 ramets). The increased abundance of *P. virgatum* in the CL region compared to the inland CHP region was best explained by both actual increased abundance and an unintended effect of the experimental design. Comparison of the chosen CL habitats did not show any difference in shade, species diversity, or abundance of exotic or invasive plants (Table 1). Weed species (e.g. *D. sanguinalis*) and invasive species (e.g. *Celastrus orbiculatus*, *Phragmites australis*) were among the most common plants, providing evidence of disturbance and/or degradation in the coastal plant communities studied.

A single-linkage dendrogram was created to represent the similarity/dissimilarity of habitat types based on observed plant taxa (Fig. 2). Hayfields and cornfields (agricultural systems) were most dissimilar to the natural or semi-managed habitat types. Core forests and edge forests showed similarity to each

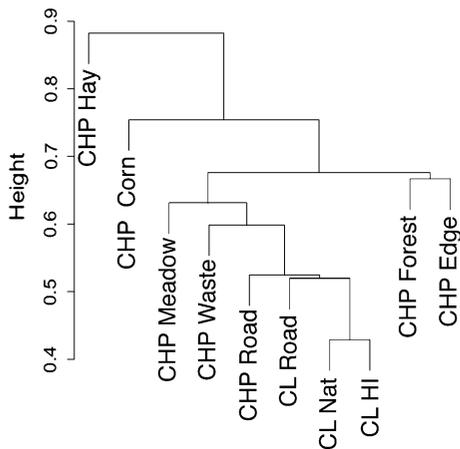


Fig. 2 Dendrogram calculated from Brays–Curtis (1957) dissimilarity, comparing all habitats in both subcoregions. Height is the dissimilarity distance. Abbreviations same as Table 1 except for: *CL Nat* CL semi-natural, *CL HI* CL human impacted

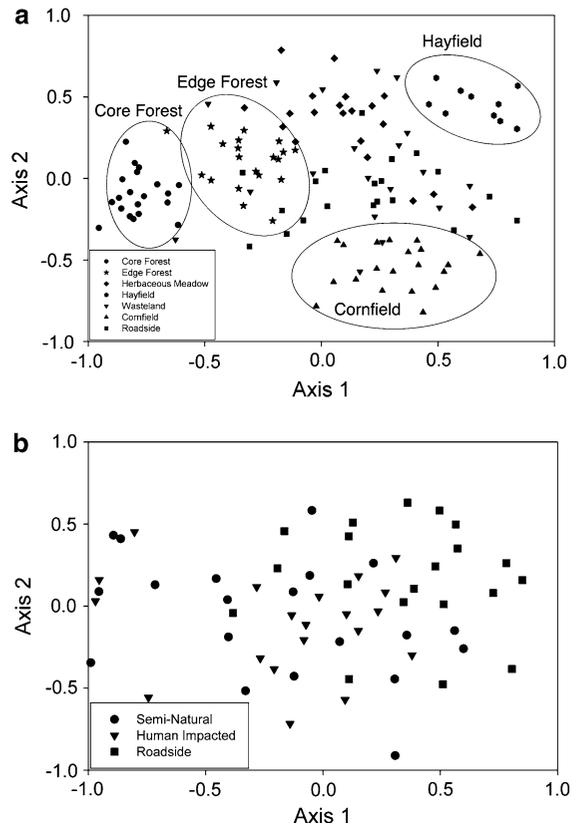


Fig. 3 Non-multidimensional scaling (NMDS) ordination plots based on dissimilarities of transects (plant community assemblages) using Brays–Curtis analysis. Circles within the plots represent clustering for habitat types. **a** CHP subcoregion with 130 transects ($r^2 = 0.70$), **b** CL subcoregion with 60 transects ($r^2 = 0.69$)

other, but dissimilarity to other habitats. Roadsides from the CHP and CL regions were closely related, as were CL semi-natural and human impacted habitats. Other data supports the similarity of CL and CHP roadside habitats due to the non-significant difference between species diversity index, % invasive ramets, % exotic plant ramets, and most common plants (*D. sanguinalis* and *Ambrosia artemisiifolia*) (Table 1). NMDS ordination plots for dissimilarity showed each transect as a discrete point and these points can be interpreted as observed plant communities (Fig. 3a–b). While no two plant communities were exactly the same, the ordination plot for CHP transects showed strong clustering in core forest and hayfield transects (Fig. 3a). Edge forest and cornfield transects showed loose clustering (Fig. 3a). The ordination plot for the coastal CL region (Fig. 3b) had no well-defined

Table 2 Bentgrasses (*Agrostis* and *Apera*) and *Panicum* species observed in habitat types in inland (CHP) and coastal (CL) subcoregions

Grass species	Common name	% CC					CHP sub-ecoregion					CL sub-ecoregion									
		Forest	Edge	Meadow	Road	Waste	Corn	Hay	Semi-nat	Human Imp	Road	Forest	Edge	Meadow	Road	Waste	Corn	Hay	Semi-nat	Human Imp	Road
<i>A. stolonifera</i>	Creeping bentgrass	12	0	35	60	55	0	10	20	20	5	5	50	0	0	0	0	0	20	20	5
<i>A. gigantea</i>	Redtop	16	0	35	55	35	0	0	15	30	5	5	50	0	0	0	0	15	30	30	5
<i>A. canina</i>	Velvet bentgrass	17	0	0	20	5	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
<i>A. capillaris</i>	Colonial bentgrass	6	0	5	20	15	0	0	15	15	0	0	15	0	0	0	0	15	15	15	20
<i>A. perennans</i>	Upland bentgrass	42	15	35	30	25	0	0	5	5	0	0	10	0	0	0	0	5	5	5	15
<i>A. scabra</i>	Rough bentgrass	2	0	0	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
<i>A. hyemalis</i>	Winter bentgrass	18	0	5	5	5	0	0	30	0	0	0	30	0	0	0	0	5	0	0	10
<i>Apera spica-venti</i>	Loose silkybent	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. virgatum</i>	Switchgrass	1	0	0	5	45	0	10	15	70	0	0	15	0	0	0	0	65	70	75	0
<i>P. rigidulum</i>	Redtop panicgrass	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>P. dichotomiflorum</i>	Fall panicgrass	9	0	5	10	50	0	95	60	5	0	0	60	0	0	0	0	5	5	30	0
<i>P. capillare</i>	Witchgrass	0	0	0	0	0	0	15	5	0	0	0	5	0	0	0	0	0	0	0	0
<i>P. amarum</i>	Bitter panicgrass	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	5	0	0

Values shown below each habitat type represent % transects containing the species. Mean % canopy cover (CC) was calculated for transects with the species

clusters, although roadside transects tended to group together.

Agrostis species

Eight species in the bentgrass group were identified and receiving populations for intraspecific and inter-specific gene flow were common (Table 2). Three species (*A. hyemalis*, *A. perennans*, and *A. scabra*) were native. The native *A. perennans* was unusual because it was the only *Agrostis* to occur in core forest habitats and tolerate higher amounts of shade (42% canopy cover compared to 0–18% for all other bentgrasses) (Table 2). Two members of the bentgrass group were not observed in this study (*Polypogon viridis* and *Polypogon monspeliensis*) although they have been included in regional floras (Dowhan 1979).

In both CL and CHP regions, transects with *Agrostis* species had a higher SDI than transects without *Agrostis* (Fig. 4). However, there were no significant differences in diversity values within each habitat type (data not shown). *Agrostis* species were more abundant in the CHP region (27.6 ramets/transect) than the CL region (12.2 ramets/transect). *Agrostis* species were most common in edge forest, meadow, roadside, and wasteland habitats which were also observed to have relatively high numbers of exotic and invasive plants (Tables 1, 2). Using a NMDS ordination plot, *Agrostis* species appear to be clustered in edge forest, herbaceous meadow, wasteland, and roadside habitats.

Attention was focused on *A. stolonifera* distribution and habitats because it has been proposed as a GE turfgrass. *A. stolonifera* was mainly associated with four habitats: edge forest, herbaceous meadow, wasteland, and roadside (Fig. 5a). *C* values calculated for the inland region revealed three *Agrostis* species as among the top ten species for co-occurrence with *A. stolonifera* (Table 3). In the CL region, *A. gigantea* had a high *C* value (0.79) for co-occurrence with *A. stolonifera* (Table 4). This provides evidence that bentgrasses occur together at a localized scale (Tables 3, 4) but proof of interspecific hybridization is impossible without molecular tools.

Panicum species

Five native *Panicum* species were observed (Table 2). Three *Panicum* species (*P. miliaceum*,

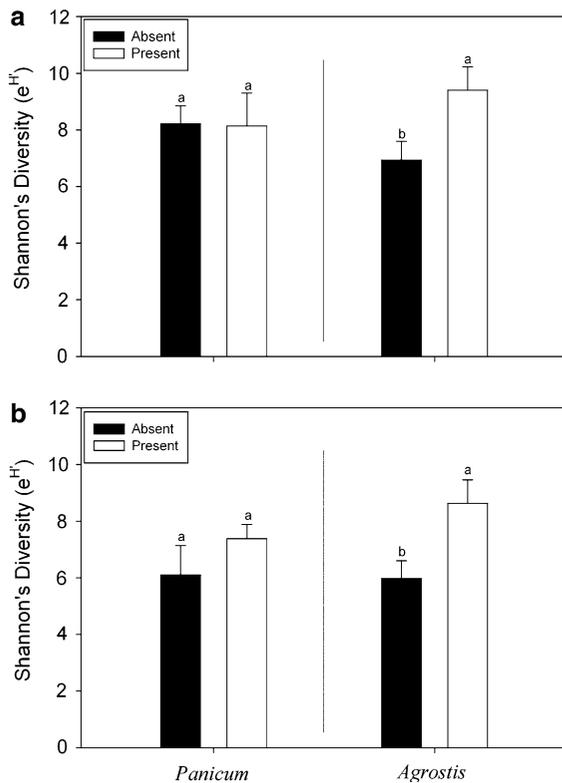


Fig. 4 Comparison of Shannon's Diversity Index for transects with and without *Agrostis* or *Panicum* species in two ecoregions. **a** CHP ecoregion, **b** CL ecoregion. Letters indicate significant difference at $P < 0.05$

P. philadelphicum, *P. verrucosum*) were not observed in this study although they have been reported in regional floras (Barkworth et al. 2007). *Panicum amarum*, which is listed as threatened or of special concern in Connecticut and Rhode Island respectively, was identified in five coastal CL transects in either semi-natural or human impacted habitats (Table 2).

In the inland CHP region, *Panicum* species were found in all habitat types except core forest. Road-sides, wasteland, and cornfields had the highest percentage of transects with *Panicum*. Fewer such transects were observed in edge forest, meadows, and hayfields (Table 2). In the CHP region, *P. virgatum* was most frequently found in roadside habitat, but it was equally distributed across the three coastal CL habitats studied (Fig. 5c–d; Table 2). In contrast, *P. dichotomiflorum* was most abundant in CHP cornfield, wasteland, and roadside habitats, but less common in coastal habitat types (Table 2).

P. capillare and *P. rigidulum* were found mainly in cornfields and herbaceous meadows, respectively. Unlike *Agrostis*, transects with *Panicum* had the same species diversity as transects without *Panicum* (Fig. 4a–b).

Analysis focused on *P. virgatum* distribution and habitats because it has been proposed as a GE biofuels feedstock crop. Receiving populations for intraspecific gene flow were observed, most notably in the CHP roadside habitats (Fig. 5c) and all CL habitats (Fig. 5d; Table 2). Furthermore, a greater abundance of *P. virgatum* was observed in the CL region than in the CHP region. Roadside habitats can be compared because of their similarity between regions; we found that *P. virgatum* had a median abundance of 42 ramets/transect ($n = 9$) in the CHP region and 84 ($n = 15$) in the CL region. This association between *P. virgatum* and roadside habitats supports an observation by Haines (2010). *P. virgatum* was relatively uncommon in CHP herbaceous meadows (5%), a surprising result because this habitat could be thought of as the closest analogue to prairies (Barkworth et al. 2007). In the CHP region, 64% of transects with *P. virgatum* also contained one or more *Panicum* species; only 30% of transects with *P. dichotomiflorum* contained *P. virgatum* (data not shown). This indicates niche overlap, but C values showed low co-occurrence (Tables 3, 4). Of the five transects with *P. amarum*, *P. virgatum* was only present in one, giving the two species a low co-occurrence value (-0.58) (Table 4).

Discussion

Patterns of grass cultivation in the U.S. will change over time due to land use patterns and climate. For example, *P. virgatum* could be planted over large areas for biofuel production, and its suitable habitat could shift northward in this century due to climate change (Barney and DiTomaso 2010). *P. virgatum* continues to receive genetic modification through selection, breeding, and engineering to create novel agronomic traits, improve adaptability, increase stress resistance, and promote efficient biofuel production. These novel traits could expand both crop range and its cultivation in marginal lands. In the case of *A. stolonifera*, golf course construction, maintenance, and abandonment are major factors in cultivation and

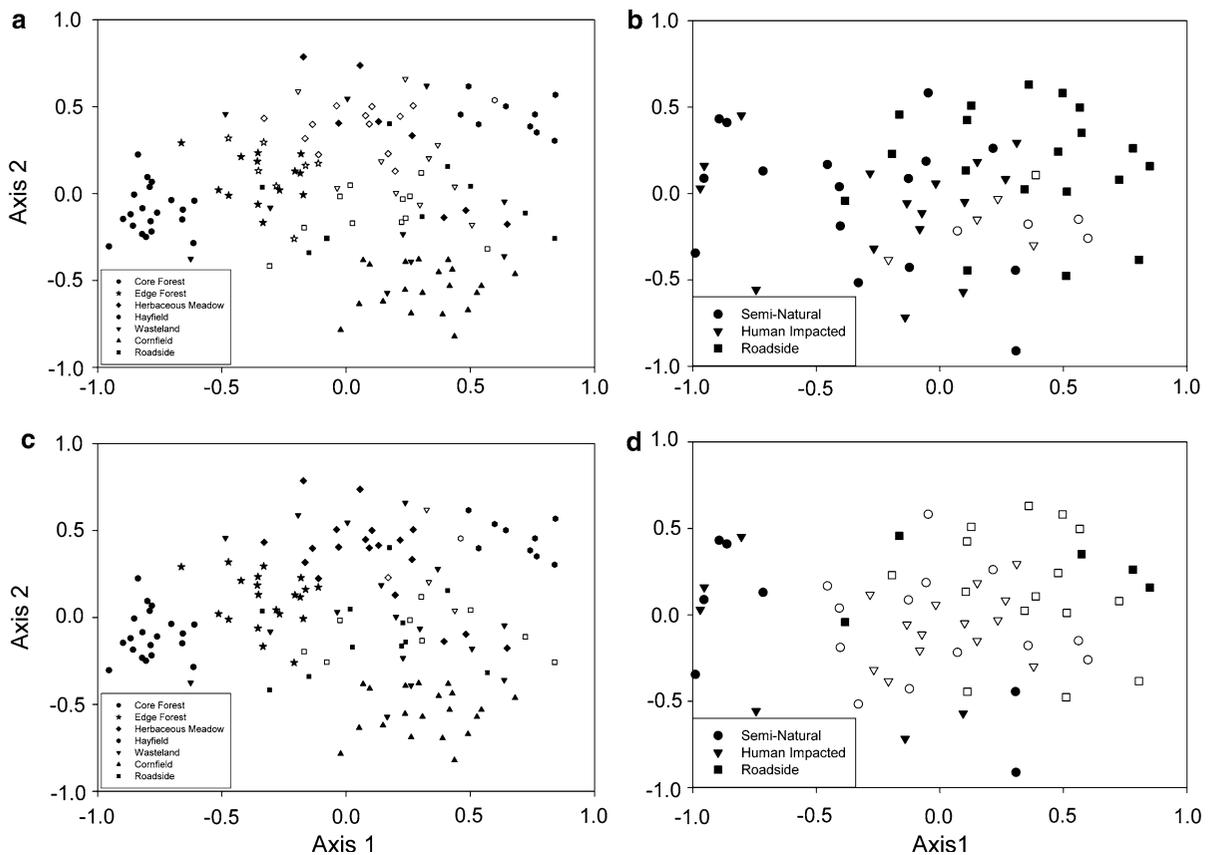


Fig. 5 Non-Multidimensional Scaling (NMDS) ordination plots for two ecoregions based on dissimilarities of transects (plant community assemblages) using Brays–Curtis analysis. **a** Outlined symbols represent *Agrostis stolonifera* in the CHP

region, **b** outlined symbols represent *A. stolonifera* in the CL region, **c** outlined symbols represent *Panicum virgatum* in the CHP region, and **d** outlined symbols represent *P. virgatum* in the CL region. Values for r^2 as shown in Fig. 3

distribution, as well as use in drainage swales, lawns, and other constructed grasslands. New traits are designed to improve turfgrass management and stress tolerance (e.g., herbicide resistance, salt tolerance) (USDA 2010). However, concerns have been expressed about future unintended consequences from pollen-mediated gene flow, the development of new weeds or invasives, and loss of genetic diversity in native grass populations (Andow and Zwahlen 2006; Stewart et al. 2003; Wilkinson and Tepfer 2009). One response to these concerns is research leading to a detailed understanding of plant species distribution, communities, habitats, and species co-occurrence that will support ERA problem formulation, tiered risk assessment research, risk management, coexistence-strategies, monitoring schemes, and exclusion zones (Wilkinson and Tepfer 2009).

We conducted field surveys to address risk assessment issues for *Agrostis* and *Panicum* grasses in the northeastern U.S. Analysis showed that the habitats studied in the inland CHP region were relatively distinct from each other with regard to plant communities, while the three coastal CL habitats overlapped (Table 1; Figs. 2, 3). *Agrostis* and *Panicum* species were not equally distributed across all habitats and ecoregions (Table 2; Fig. 5a–d). The higher biodiversity in plant communities with *A. stolonifera* may be due to the increased presence of exotic species (Fig. 4). A small positive correlation was found between the number of *A. stolonifera* ramets and the number of exotic ramets in the transect (slope = 0.63, $r^2 = 0.13$, data not shown). Our results are consistent with the idea that increased local species diversity is often associated with biotic homogenization, especially in habitats altered by anthropogenic forces

Table 3 Plant species co-occurring with *Agrostis stolonifera* and *Panicum virgatum* in the inland CHP subcoregion

<i>Agrostis stolonifera</i>	C value	<i>Panicum virgatum</i>	C value
<i>Agrostis stolonifera</i> ^c	1.00	<i>Panicum virgatum</i>	1.00
<i>Agrostis gigantea</i> ^c	0.39	<i>Poa trivialis</i> ^c	0.77
<i>Symphotrichum ericoides</i> ^a	0.37	<i>Bromus tectorum</i> ^{b,c}	0.74
<i>Potentilla simplex</i> ^a	0.35	<i>Polygonum hydropiper</i> ^c	0.73
<i>Juncus</i> spp.	0.28	<i>Polygonum careyi</i>	0.73
<i>Agrostis capillaris</i> ^c	0.26	<i>Apera spica-venti</i> ^c	0.72
<i>Agrostis canina</i> ^c	0.26	<i>Anthemis arvensis</i> ^{a,c}	0.71
<i>Dichanthelium sphaerocarpon</i> ^{a,c}	0.24	<i>Eupatorium perfoliatum</i>	0.71
<i>Schizachyrium scoparium</i>	0.24	<i>Saponaria officinalis</i> ^c	0.71
<i>Chaenorhinum minus</i> ^{a,c}	0.22	<i>Medicago sativa</i> ^c	0.71
<i>Setaria faberi</i> ^c	−0.47	<i>Panicum dichotomiflorum</i> ^a	−0.14
<i>Thelypteris noveboracensis</i>	−0.48	<i>Ambrosia artemisiifolia</i> ^a	−0.16
<i>Quercus rubra</i>	−0.51	<i>Vaccinium corymbosum</i>	−0.17
<i>Vaccinium corymbosum</i>	−0.54	<i>Solidago rugosa</i>	−0.30
<i>Acer rubrum</i>	−0.72	<i>Acer rubrum</i>	−0.60

The 10 species with highest C values for co-occurrence are shown, as well as the five species least likely to co-occur

^a Listed as a weed in Weeds of the Northeast (Uva et al. 1997)

^b Listed as an invasive plant by CT Invasive Plant Council (http://nbi-nin.ciesin.columbia.edu/ipane/ctcouncil/CT_invasive.htm)

^c Listed as an exotic species (USDA plants database, <http://plants.usda.gov/>)

Table 4 Plant species co-occurring with *Agrostis stolonifera* and *Panicum virgatum* in the coastal CL subcoregion

<i>Agrostis stolonifera</i>	C value	<i>Panicum virgatum</i>	C value
<i>Agrostis stolonifera</i> ^c	1.00	<i>Panicum virgatum</i>	1.00
<i>Rubus pensilvanicus</i>	0.79	<i>Celastrus orbiculatus</i> ^{a,b,c}	0.30
<i>Agrostis gigantea</i> ^c	0.79	<i>Toxicodendron radicans</i> ^a	0.22
<i>Poa palustris</i>	0.79	<i>Euthamia graminifolia</i>	0.21
<i>Secale cereale</i> ^c	0.79	<i>Ambrosia artemisiifolia</i> ^a	0.21
<i>Sonchus oleraceus</i> ^{a,c}	0.79	<i>Achillea millefolium</i> ^a	0.14
<i>Amelanchier canadensis</i>	0.79	<i>Baccharis halimifolia</i>	0.11
<i>Boehmeria cylindrica</i>	0.79	<i>Digitaria sanguinalis</i> ^a	0.09
<i>Agalinis tenuifolia</i>	0.79	<i>Schizachyrium scoparium</i>	0.06
<i>Lespedeza intermedia</i>	0.79	<i>Dichanthelium clandestinum</i>	0.04
<i>Vicia sativa</i> ^{a,c}	0.79	<i>Phragmites australis</i> ^{a,b,c}	0.02
<i>Ammophila breviligulata</i>	0.22	<i>Solidago sempervirens</i>	−0.55
<i>Lathyrus japonicus</i>	0.01	<i>Panicum amarum</i>	−0.58
<i>Spartina patens</i>	−0.02	<i>Ammophila breviligulata</i>	−0.64
<i>Panicum virgatum</i>	−0.07	<i>Lathyrus japonicus</i>	−0.71
<i>Solidago sempervirens</i>	−0.07	<i>Spartina patens</i>	−0.72

The 10 species with highest C values for co-occurrence are shown, as well as the five species least likely to co-occur

^a Listed as a weed in Weeds of the Northeast (Uva et al. 1997)

^b Listed as an invasive plant by CT Invasive Plant Council (http://nbi-nin.ciesin.columbia.edu/ipane/ctcouncil/CT_invasive.htm)

^c Listed as an exotic species (USDA plants database, <http://plants.usda.gov/>)

(Smart et al. 2006). Co-occurrence of exotic, weedy *Agrostis* species that can hybridize was common (Table 3). Overall, it was clear that receiving populations for pollen-mediated gene flow were common and distributed across the natural, cultural, and agricultural habitats studied. The observation of one *Panicum* species with special protection status raises questions about the possibility of interspecific gene flow leading to harm. However, there is no proof of interspecific gene flow with *P. virgatum*, so the importance of co-occurrence for pollen-mediated gene flow is impossible to assess at this time.

Our results show that both *Agrostis* and *Panicum* species most commonly occur in habitats that have moderate to high human management and/or disturbance events. The diagram in Fig. 6 ranks habitat types in each ecoregion along a continuum for disturbance and management. While somewhat subjective, this diagram promotes consideration of the likelihood and potential effects of management or disturbance events (e.g. herbicide application, winter deicing salt, irrigation) that could favor or discourage grasses with novel traits (e.g. herbicide tolerance, salt tolerance, drought tolerance). This type of diagram could guide tiered risk assessment protocols for regulatory science. In our study, *Agrostis* species were most common in CHP habitats with intermediate management or disturbance regimes, while habitats at both ends of the spectrum (core forests, hayfields, cornfields) had few or no *Agrostis*. In contrast, there was no difference in *Agrostis* distribution between semi-natural, roadside, and human impacted habitats in the CL region.

While *P. virgatum* was observed in semi-natural coastal habitat, it was similar to *Agrostis* in that it

also occurred in disturbed or managed habitats (e.g. CHP roadsides, CL roadsides, CL human impacted habitats) (Table 2; Fig. 5). This was somewhat surprising since *P. virgatum* is native to high salt marshes along the coast. Studies of the invasive grass *P. australis* showed that spread was facilitated by optimal growing conditions along roadsides and other rights-of-way (Jodoin et al. 2008; Maheu-Giroux and de Blois 2007). These areas functioned as linear wetland networks with anthropogenic disturbance that assisted *Phragmites* dispersal. In our study, *P. australis* was in the ‘top ten’ vascular plants most likely to co-occur with *P. virgatum* in the coastal CL region (Table 4). Thus, roadside habitats could function as linear networks facilitating the spread of *P. virgatum*, *Phragmites*, and other species. Irrigation ditches, powerline rights-of-way, and railroad rights-of-way have also shown function as corridors for grass dispersal (Ahrens et al. 2011; Mallory-Smith and Zapiola 2008). The function of these linear corridors should be studied prior to the release of GE grasses because retraction of transgenes is nearly impossible and they can persist without selection pressure (Marvier and Van Acker 2005; Warwick et al. 2007; Zapiola et al. 2008). Information about dispersal mechanisms, in combination with knowledge about plant community assemblages and plant species distribution, will provide a strong basis for ecological risk assessments, coexistence-strategies, monitoring, and geographic exclusion zones.

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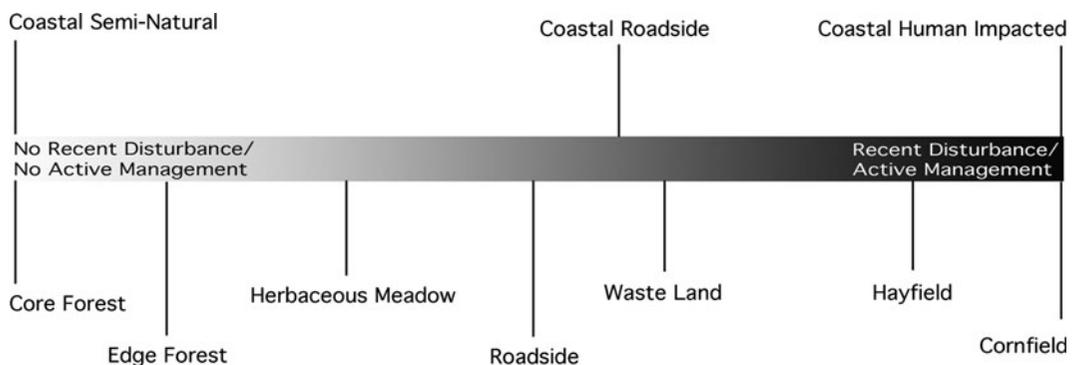


Fig. 6 Habitat types arranged according to degree of disturbance events and management activities. Habitats shown above the line were studied in the coastal CL region and habitats below the line occurred in the inland CHP region

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