RESEARCH ARTICLE

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Bud-bank and tiller dynamics of co-occurring C_3 caespitose grasses in mixed-grass prairie[1]

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PREMISE OF THE STUDY: Tiller recruitment from the belowground bud bank of caespitose grasses influences their ability to monopolize local resources and, hence, their genet fitness. Differences in bud production and outgrowth among tiller types within a genet and among species may explain co-occurrence of caespitose grasses. This study aimed to characterize genet bud-bank and tiller production and dynamics in two co-occurring species and compare their vegetative reproductive strategies.

METHODS: Bud-bank and tiller dynamics of *Hesperostipa comata* and *Nassella viridula*, dominant C_3 caespitose grasses in the northern mixed-grass prairie of North America, were assessed throughout an annual cycle.

KEY RESULTS: The two species showed similar strategies, maintaining polycyclic tillers and thus creating mixed-age genet bud banks comprising multiple bud cohorts produced in different years. Vegetative tillers produced the majority of buds, whereas flowering tillers contributed little to the bud bank. Buds lived for at least 2 yr and were maintained in multiple developmental stages throughout the year. Because bud longevity rarely exceeded tiller longevity, tiller longevity drove turnover within the bud bank. Tiller population dynamics, more than bud production per tiller, determined the differential contribution of tiller types to the bud bank. *Nassella viridula* had higher bud production per tiller, a consistent annual tiller recruitment density, and greater longevity of buds on senesced and flowering tillers than *H. comata*.

CONCLUSIONS: Co-occurring C_3 caespitose grasses had similar bud-bank and tiller dynamics contributing to genet persistence but differed in bud characteristics that could affect genet longevity and species coexistence.

KEY WORDS bud bank; caespitose; genet longevity; grasses; *Hesperostipa comata*; mixed-grass prairie; *Nassella viridula*; polycyclicity; tiller dynamics; vegetative reproduction

The ecological success of the caespitose (bunchgrass) growth form is attributed to its effective monopolization of local soil resources. Resource monopolization is achieved by sharing resources through physiological integration within ramet hierarchies, regulating ramet natality and mortality via density-dependent growth, and consolidating pools of soil resources (e.g., soil organic carbon and total nitrogen) beneath the genet (Derner et al., 1997; Briske and Derner, 1998; Derner and Briske, 1998). Although tiller natality and mortality can exhibit dramatic fluctuations, resulting in a highly dynamic tiller population, caespitose genet persistence depends on maintaining a stable or increasing tiller population via consistent tiller recruitment from a reserve of belowground buds ("bud bank," *sensu* Harper, 1977).

In perennial grasslands, most tiller recruitment occurs from belowground buds rather than from seeds. Seedling recruitment is rare in the dominant grasses across the Great Plains (Fair et al., 1999; Peters, 2000; Benson and Hartnett, 2006). For example, >99% of all established stems in both burned and unburned tallgrass prairie communities were recruited from the bud bank (Benson and Hartnett, 2006). Vegetative recruitment was also more common than seed recruitment in the shortgrass steppe of northern Colorado, where the seed bank primarily consisted of annuals rather than the dominant perennials (Coffin and Lauenroth, 1989).

Bud banks play a critical role in perennial grassland function and structure. Bud and tiller demography can introduce lag effects into aboveground net primary production of grassland (Ott and Hartnett, 2012a; Reichmann and Sala, 2014). Belowground bud

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banks stabilize grassland communities during grazing and short-term drought (Dalgleish and Hartnett, 2009; VanderWeide and Hartnett, 2015). Clonal traits, such as bud banks, also influence plant community assembly and composition (Klimesova and Klimes, 2008; Rusch et al., 2011).

Bud production is fundamentally tied to tiller growth and development (Sharman, 1942; Langer, 1972; for illustrations, see Etter, 1951; Briske, 1991). Grass tillers (ramets) are composed of multiple stacked phytomers, which are modular units, each composed of a leaf blade, a sheath, and an axillary bud at the node. During vegetative growth, phytomers maintain very short internodes such that buds remain at or below ground level. Thus, when a grass tiller adds a new leaf, it usually adds a belowground bud. A genet can be composed of tillers of different cohorts, developmental stages, and reproductive status (flowering or nonflowering). Bud production and dormancy can vary among tiller types. For example, flowering tillers of *Andropogon gerardii* produce more buds per tiller than its vegetative tillers (Ott and Hartnett, 2011). Because the genet bud bank comprises all buds produced on each of its tillers, a genet's bud-bank characteristics are determined by its tiller composition and the bud production of each tiller type.

Coexisting perennial grass species can vary greatly in life-history characteristics such as longevity, sexual reproductive effort, dispersal, and patterns of vegetative reproduction and growth (O'Connor, 1991). Studies focusing on the basic patterns of vegetative reproduction and growth of grass species have examined aboveground vegetative-tiller recruitment of controlled plantings (Langer, 1956; Robson, 1968; Colvill and Marshall, 1984) and natural populations of single or coexisting species (Langer et al., 1964; Jónsdóttir, 1991; Herben et al., 1993; Janisova, 2006). A few studies in tallgrass prairie have incorporated the relationship of belowground bud dynamics to aboveground tiller dynamics when examining the vegetative reproductive characteristics of coexisting perennial grass species (Dalgleish et al., 2008; Ott and Hartnett, 2012a). Two co-occurring grasses in mesic tallgrass prairie, the dominant rhizomatous C_4 grass *A. gerardii* and the subdominant caespitose C_3 grass *Dichanthelium oligosanthes*, differed in their bud production, bud development, bud longevity, and tiller phenology (Ott and Hartnett, 2012a). The differences in bud-bank characteristics of these two species were likely due to their differences in growth form (i.e., rhizomatous vs. caespitose) and photosynthetic pathway (C_3 vs. C_4). Additional studies of the bud and tiller dynamics of co-occurring grasses are needed to ascertain the role of belowground bud banks in genet persistence and fitness, and to determine whether variation in vegetative reproduction and bud-bank traits facilitates their coexistence.

Hesperostipa comata and *Nassella viridula* are dominant C_3 caespitose grasses in the northern Great Plains region of North America. These co-occurring species provide an opportunity to compare the vegetative reproductive characteristics of two dominant grasses in mixed-grass prairie, which can be compared with previous work conducted in tallgrass prairie. Because *H. comata* and *N. viridula* have similar growth forms and photosynthetic pathways, any variation in vegetative reproduction would be due to species differences. The objective of the present study was to quantify bud-bank and tiller dynamics throughout an annual cycle of the two co-occurring perennial grasses *H. comata* and *N. viridula* to (1) characterize the bud-bank dynamics and vegetative life-history patterns of two dominant C_3 caespitose grasses; (2) determine whether tillers of varying age, flowering status, and photosynthesizing status differentially contribute to the genet bud bank; and (3) identify whether these two species have different vegetative reproductive characteristics that may affect their genet growth and persistence or their coexistence.

MATERIALS AND METHODS

***Site description*—**The study was conducted at Wind Cave National Park, a 13 699-ha mixed-grass prairie interspersed with ponderosa pine forest located at the southeastern extent of the Black Hills in western South Dakota (43°33′N, 103°29′W). The vegetation is dominated by cool-season C_3 grasses such as *H. comata*, *Pascopyrum smithii*, and *N. viridula*, with discrete patches of less abundant warm-season C_4 grasses including *A. gerardii*, *Bouteloua curtipendula*, and *B. gracilis*. Bison (*Bison bison*), elk (*Cervus elaphus*), pronghorn (*Antilocapra americana*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*O. hemionus*) are the major large herbivores. Bison and elk are the primary consumers of grasses, owing to their grazing habits and population sizes. The region's semiarid climate has cool winters (average January temperature: −2.3°C) and warm summers (average July temperature: 22.9°C), with moderate rainfall (499 mm) primarily occurring from April through October, and especially in May and June. During our study, annual precipitation at Wind Cave National Park in 2010 and 2011 was 646 mm and 575 mm, respectively. April, May, and June 2010 and May 2011 had ≥58 mm of rainfall above the long-term average for each month. Wind Cave National Park has a hilly topography (elevation range: 1113–1527 m).

The northern portion of the 42-ha fenced Elk Mountain enclosure within Wind Cave National Park was used for our study. The enclosure excludes bison but not other herbivores. Prescribed fire occurs every 5–7 yr, and the Elk Mountain enclosure was last burned in fall 2008. The enclosure is at an elevation of 1310 m, and the prairie primarily has loamy-skeletal soils (Typic Argiustolls) but includes a small area with fine-loamy soil (Fluventic Haplustolls; Natural Resources Conservation Service, 2013).

***Field sampling*—**In June and July 2010, we established 10 sites separated by ≥50 m within the grassland portion of the Elk Mountain enclosure. At each site, populations of *H. comata* and *N. viridula* were located and 14 individuals of each species were randomly selected and marked using a metal tag and a wire ring. Individuals were never heavily grazed during the study. *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needle-and-thread grass) and *N. viridula* (Trin.) Barkworth (green needlegrass) are both caespitose perennial C_3 grasses. *Hesperostipa comata* populations are often composed of individuals with small basal areas and are located in drier sites within the community than *N. viridula* (Redmann, 1975). *Nassella viridula* individuals tend to have large basal areas with substantial biomass once established (Rogler, 1960). Although each species is palatable to grazers, *N. viridula* declines with heavy grazing whereas *H. comata* resists grazers by retaining sharp, needle-like florets (Larson and Johnson, 1999).

Because of their caespitose growth form, discrete genets (i.e., individuals) of *H. comata* and *N. viridula* are easy to identify. Basal areas of *H. comata* and *N. viridula* genets were determined by taking two perpendicular caliper measurements at the base of each individual and calculating the area of the ellipse. The 2010 flowering tillers of each individual were marked using small wire rings

and counted. Beginning on 20 August 2010, one individual of each species from each site was harvested to a 7-cm depth approximately every 3 wk during the growing season (i.e., while soil temperatures remained consistently above freezing; Ott, 2014) and washed to remove soil. Sampling occurred on 14 sampling dates over 15 mo, with the final harvest occurring on 4 November 2011. Although *N. viridula* was harvested every sampling date, samples from only nine sampling dates were analyzed in the laboratory because of time constraints. Therefore, a total of 140 *H. comata* and 90 *N. viridula* individuals were analyzed.

***Laboratory analysis*—**Bud and tiller development is progressive. Tillers were distinguished from buds by their elongation in relation to the prophyll. Buds were contained within the prophyll, and tillers had elongated past the prophyll. For the present study, two bud stages and seven tiller stages were defined (Table 1 and Fig. 1). Live buds were divided into two size classes: small (B_1) and large (B_2). Large buds transitioned into small juvenile tillers (VT_1) and subsequently large juvenile tillers (VT_2). Juvenile tillers ($VT_{1,2}$) typically had not emerged aboveground. Therefore, buds and juvenile tillers were considered collectively as "potential tiller recruits." Large juvenile tillers emerged aboveground as small vegetative tillers (VT_3) and grew into large vegetative tillers (VT_4). Vegetative tillers ($VT_{3,4}$) could either flower (FT) or senesce (ST). Flowering and tiller senescence result in the decay and loss of the aboveground portion of the tiller, but the belowground tiller base could persist. Belowground bases that have lost the aboveground portion of their tiller were considered residual tillers (RT).

Buds and tillers from each individual plant were examined using a dissecting scope with magnifications between 7× and 40×. Tillers were counted, assessed to be living or dead, and classified by size class and flowering status. During the study, live tillers were found to belong to multiple generations. Therefore, aboveground tillers (including $VT_{3,4}$, FT, and ST) were further classified according to generation (primary/1, secondary/2, tertiary/3, quaternary/4, quinary/5; Welker et al., 1987). In a sequence of tillers growing directly from one another, generation was assigned from oldest to youngest. For example, in a series of three attached tillers, the oldest tiller is considered the primary tiller and the youngest is the tertiary tiller. At the beginning of the study, tillers attached to residual tillers (RT) were considered primary tillers even though all these primary tillers may not have been recruited at the same time. Using this classification of primary tillers enabled us to track generational tiller dynamics. Our frequent sampling ensured that we detected whether and when primary tillers were senescing (i.e., transitioning to ST) and enabled proper generation-classification for each aboveground tiller throughout the study. Hereafter, tillers may be referred to by their generation, if appropriate, and symbol (e.g., primary small vegetative tillers [$1VT_3$], secondary vegetative tillers [$2VT_{3,4}$], juvenile tillers [$VT_{1,2}$], tertiary flowering tiller [3FT]; Fig. 1).

As a grass tiller grows, it adds buds to its base beneath the soil. The number and longevity of these buds may depend on the tiller that produces them (e.g., Ott and Hartnett, 2011). Although all aboveground tillers were counted and classified for the entire individual, buds and juvenile tillers were counted and classified on a subsample of five randomly selected tillers of each combination of generation and flowering-status classification (exception: 10 tillers were used to assess buds and juvenile tillers on residual tillers). Dead buds were identified by their soft, spongy, or mealy brown interiors and were easily distinguished from live buds. For the first sample date (20 August 2010), small juvenile tillers (VT_1) were counted as large juvenile tillers (VT_2).

***Statistical analyses*—**Seven response variables—including total live and dead tillers ($VT_{3,4}$, FT, ST, RT) per basal area, $VT_{3,4}$ per basal area, ST per basal area, $B_{1,2}$ per basal area, $VT_{1,2}$ per basal area, total buds and juvenile tillers ($B_{1,2}$, $VT_{1,2}$) per vegetative tiller ($VT_{3,4}$),

TABLE 1. Developmental bud and tiller stages as classified for our study of the C_3 caespitose grasses *Hesperostipa comata* and *Nassella viridula*.

Symbol	Developmental Stage	Description
B_1	Small buds	<3.0 mm height
B_2	Large buds	≥3.0 mm height
VT_1	Small juvenile tillers	Apex elongated <3.0 mm past prophyll
VT_2	Large juvenile tillers	Elongated 3.0 mm to 4.0 cm past prophyll (*H. comata*); elongated 3.0 mm to 5.0 cm past prophyll (*N. viridula*)
VT_3	Small vegetative tillers	4.0–15.0 cm height (*H. comata*) 5.0–18.9 cm height (*N. viridula*)
VT_4	Large vegetative tillers	>15.0 cm height (*H. comata*) >18.9 cm height (*N. viridula*)
FT	Flowering tillers	
ST	Senesced tillers	Aboveground parts senesced but retaining live residual base with buds
RT	Residual tillers	Aboveground parts absent but retaining residual base with buds

Notes: Shaded developmental stages occur belowground. Juvenile tillers (VT_1, VT_2) were <9% of the recorded vegetative tiller height for each species (Great Plains Flora Association, 1986). Small and large vegetative tillers were smaller and larger than 33% of the recorded height, respectively.

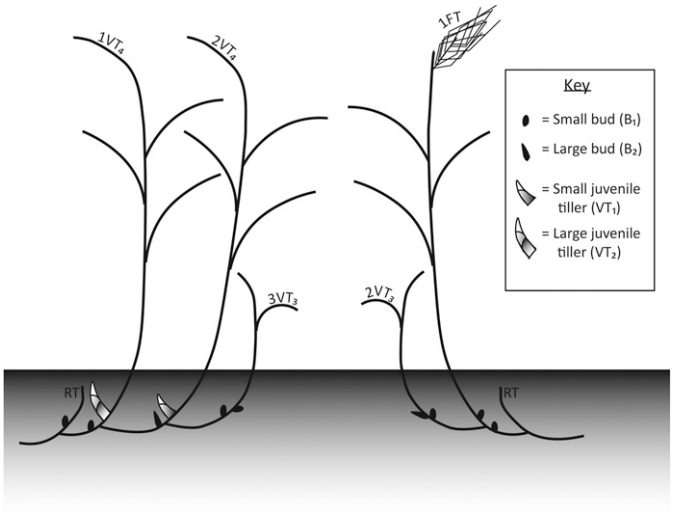

FIGURE 1 Conceptual diagram of tiller classifications and their associated propagules (RT = residual tiller, VT_3 = small vegetative tiller, VT_4 = large vegetative tiller, and FT = flowering tiller). See Table 1 for further descriptions of bud and tiller stages. Numbers preceding notation of vegetative and flowering tillers indicate the tiller generation. For example, the secondary large vegetative tiller ($2VT_4$) originated from a propagule on the primary large vegetative tiller ($1VT_4$), which originated from a propagule on the residual tiller (RT). Note that distances between tillers are lengthened in this diagram to more easily depict individual tillers. Because both species are bunchgrasses, individual tillers in the field are situated in tight clusters with short internodes between them.

and total buds and juvenile tillers per ST—were evaluated using a two-way factorial treatment structure with the factors of date and species in a randomized complete block design (RCBD) blocking on site (PROC MIXED, SAS version 9.2; SAS Institute 2008). Bud production was reported per tiller and per basal area (i.e., density) to examine bud production differences at both the tiller and genet levels. First, a multivariate analysis of variance (MANOVA) including all seven response variables was conducted to test for overall treatment effects before proceeding with univariate analysis of each response variable (PROC GLM, SAS). Although there was a departure from multivariate normality, assumptions were deemed satisfactory enough to run a MANOVA. Significant differences among dates and species exist for some of the seven response variables (MANOVA, Wilks's $\lambda = 0.016$, $F_{147, 958.7} = 5.59$, $P < 0.0001$). Total buds and juvenile tillers per 2011 flowering tiller was analyzed similarly but separately from these seven variables. Because *N. viridula* did not undergo laboratory analysis for every sampling date, each univariate analysis was analyzed with missing treatment combinations and alternate main effects and interaction contrasts according to Milliken and Johnson (2009).

To investigate generation effects within each species, $VT_{3,4}$ density and ST density were evaluated using a two-way factorial treatment structure with the factors of date and generation in an RCBD blocking on site with a split-plot (PROC MIXED, SAS). The factor of date was applied to the whole-plot experimental unit of individual genet, and the factor of generation was applied to the subplot experimental unit of tiller. The Kenward-Roger method was used to approximate the denominator degrees of freedom. Contrasts compared the density of $1VT_{3,4}$ versus $2VT_{3,4}$ for each species in both fall 2010 and fall 2011. Total buds and juvenile tillers per $VT_{3,4}$ were evaluated using a three-way factorial treatment structure with the factors of species, date, and generation in RCBD blocking on site with a split-plot using the Kenward-Roger method (PROC MIXED, SAS). The factors of species and date were applied to the whole-plot experimental unit of individual genet, and the factor of generation was applied to the subplot experimental unit of tiller. Because *N. viridula* did not undergo laboratory analysis for every sampling date and tertiary tillers did not occur on every sampling date, the data were analyzed with missing treatment combinations and alternate main effects and interaction contrasts according to Milliken and Johnson (2009). Three contrasts compared the potential tiller recruits of each species for each generation, averaged over dates on which both species were sampled. Four contrasts compared bud and juvenile tiller production per $VT_{3,4}$ of primary to secondary $VT_{3,4}$ and of secondary to tertiary $VT_{3,4}$ within each species. Applying Bonferroni multiple comparison adjustments, these contrasts were considered significant if $P < 0.007$.

Mean individual basal area was evaluated using species (2-levels) as the treatment factor in a randomized RCBD blocking on site, using Satterthwaite's degrees-of-freedom approximation and a separate variance for each species (PROC MIXED, SAS).

RESULTS

***Hesperostipa comata*—**Vegetative-tiller ($VT_{3,4}$) density decreased significantly during summer 2011 as a turnover between tiller generations occurred (Fig. 2C). Primary tillers had a significantly greater density in fall 2010 than secondary tillers (contrast, $F_{1, 245} = 22.59$, $P < 0.0001$), but secondary tillers had a significantly greater density in fall 2011 than primary tillers (contrast, $F_{1, 245} = 7.27$, $P = 0.008$; Fig. 3A). A large majority of primary vegetative tillers were in the large size class throughout the year, but secondary vegetative tillers were primarily in the small size class until they transitioned to the large size class when genets flowered in the spring (Appendix S1; see Supplemental Data with the online version of this article). Only 13.9 ± 1.5% ($n = 39$) of vegetative tillers ($VT_{3,4}$) flowered, and 96.2 ± 1.7% ($n = 50$) of flowering tillers were primary generation tillers.

Bud and juvenile tiller production (i.e., potential tiller recruits) varied with tiller generation and size. Overall, primary vegetative tillers maintained significantly more potential tiller recruits than secondary vegetative tillers (contrast, $F_{1, 313} = 20.24$, $P < 0.0001$), and secondary vegetative tillers maintained significantly more potential tiller recruits than tertiary vegetative tillers (contrast, $F_{1, 360} = 18.68$, $P < 0.0001$). These differences were especially evident when tiller generations differed in size. The strong increase in potential tiller recruits per vegetative tiller in July (Fig. 4A) mainly resulted from secondary tillers transitioning from the small to the large size class (Appendix S1).

The majority of a genet's potential tiller recruits (~90%) were borne on vegetative tillers ($VT_{3,4}$; Fig. 5A). Potential tiller recruits on 2011 flowering tillers (FT) rapidly senesced 6 wk after flowering (Fig. 4B). Once a vegetative tiller senesced, its potential tiller recruits either transitioned to small vegetative tillers or senesced (Fig. 4C). Residual tillers and older 2010 flowering tillers contributed few or no potential tiller recruits to the genet (averages: 0.011 ± 0.007 buds and juvenile tillers per RT, $n = 137$; 0.07 ± 0.02 buds and juvenile tillers per 2010 FT, $n = 132$). Small buds made up at least 78 ± 1% of the supply of potential tiller recruits throughout the year, and their contribution increased to 92 ± 1% between May and August 2011 (Fig. 5A). Large buds and juvenile tillers were present in small amounts throughout the year (Fig. 5A) but were never found on 2011 flowering tillers and were rarely observed on senesced and tertiary vegetative tillers.

***Nassella viridula*—**Owing to generational vegetative-tiller dynamics, vegetative-tiller ($VT_{3,4}$) density fluctuated during the annual cycle. Overall vegetative-tiller density decreased significantly in fall 2010 as a result of primary tiller senescence, but increased in spring 2011 as a result of secondary and tertiary tiller production and primary tiller renewal (Figs. 2D and 3B). Primary tillers had significantly greater density in fall 2010 than secondary tillers (contrast, $F_{1, 160} = 14.53$, $P = 0.0002$), but secondary tillers had significantly greater density in fall 2011 than primary tillers (contrast, $F_{1, 160} = 16.30$, $P < 0.0001$; Fig. 3B). The temporary increase in primary vegetative-tiller density in early May until early summer was due to a brief renewal of primary senesced tillers to photosynthesizing status (i.e., $1VT_{3,4}$) as their growing points had not fully senesced along with their aboveground leaves over the winter (Fig. 3B). Among all live vegetative tillers, 11.8 ± 1.3% flowered ($n = 40$) and 97.6 ± 1.2% ($n = 38$) of flowering tillers were from primary tillers. When vegetative tillers were recruited to flower, the majority of secondary tillers transitioned from the small to the large size class (Appendix S1).

Tiller characteristics, especially generation and size, affected bud and juvenile tiller production (i.e., potential tiller recruits) of *N. viridula*. Primary vegetative tillers produced significantly more potential tiller recruits than secondary tillers (contrast, $F_{1, 305} = 86.5$,

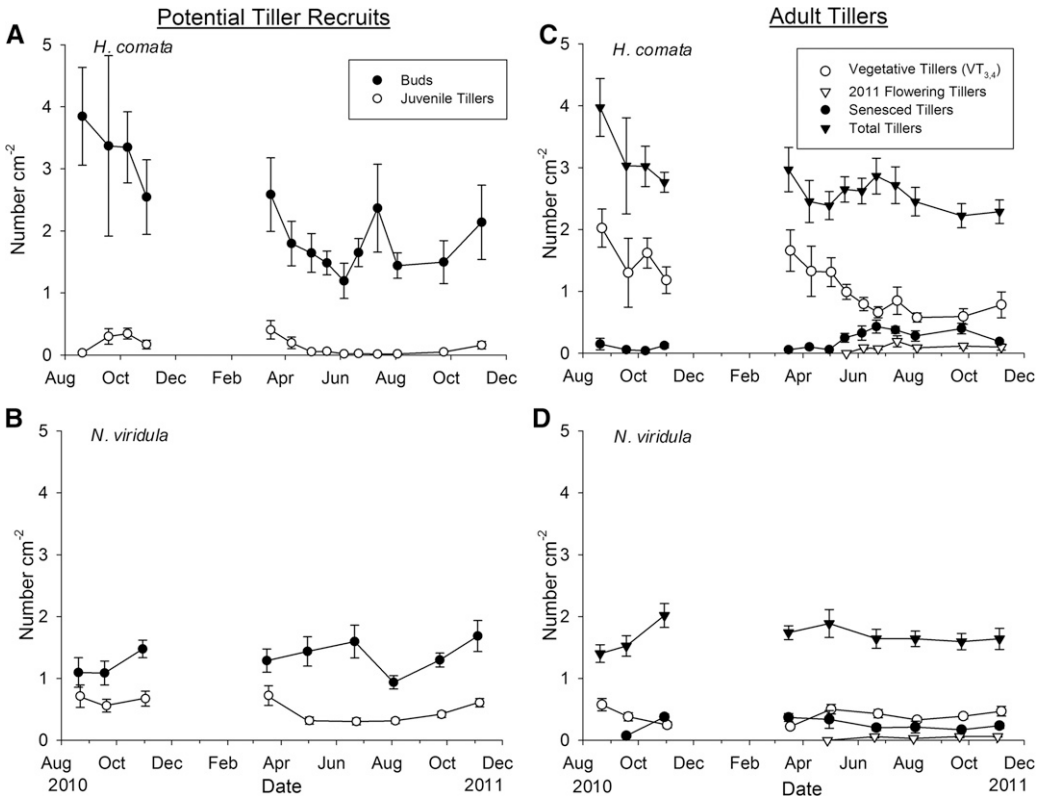

FIGURE 2 (A, B) Potential tiller recruits and (C, D) adult tillers per genet basal area (cm^2) in *Hesperostipa comata* and *Nassella viridula*. Main and interaction effects of date and species for these variables are presented in Table 2. Total tillers include all vegetative tillers ($VT_{3,4}$), flowering tillers (FT), senesced tillers (ST), and residual tillers (RT). Error bars are ±SE.

$P < 0.001$), and secondary tillers produced significantly more potential tiller recruits than tertiary tillers (contrast, $F_{1, 334} = 65.5$, $P < 0.0001$ (Appendix S2; see Supplemental Data with the online version of this article). Vegetative tillers maintained a greater number of potential tiller recruits in late summer and fall because the majority of vegetative tillers were large (i.e., VT_4; Appendix S1).

Within a genet, live tillers were the main source of potential tiller recruits (Fig. 5B). However, senesced tillers maintained ~40% of the overwintering genet supply of potential tiller recruits (Fig. 5B). In the spring, potential tiller recruits on senesced tillers rapidly senesced or transitioned to vegetative tillers (Fig. 4C). Residual tillers had few or no potential tiller recruits (average: 0.033 ± 0.010 buds and juvenile tillers per RT, $n = 90$). Flowering tillers contributed small amounts to the potential tiller recruit supply for up to 1 yr following flowering (Fig. 5B). However, few or none of these potential tiller recruits transitioned to vegetative tillers (Fig. 4B). All bud and juvenile tiller developmental stages occurred throughout the annual cycle of *N. viridula* and occurred on vegetative, flowering, and senesced tillers of every generation (Fig. 5B). Approximately half (57.7 ± 1.3%) of a genet's supply of potential tiller recruits consisted of small buds throughout the year (Fig. 5B). Large buds and juvenile tillers were the remaining half (42.3 ± 1.3%) of the supply of potential tiller recruits.

***Comparison of* H. comata *and* N. viridula**—*Nassella viridula* produced a greater number of potential tiller recruits per tiller than *H. comata* on vegetative, flowering, and senesced tillers (Fig. 4). Production of potential tiller recruits was also significantly higher on primary and secondary vegetative tillers of *N. viridula* than on comparable tillers of *H. comata* (Appendix S2; contrast, primary: $F_{1, 439} = 148.8$, $P < 0.0001$; secondary: $F_{1, 441} = 43.1$, $P < 0.001$; tertiary: $F_{1, 484} = 5.10$, $P = 0.024$ [nonsignificant according to Bonferroni]). Bud density was significantly greater in *H. comata* than in *N. viridula* in the 2010 growing season, but not in the 2011 growing season (Fig. 2A, B). Juvenile tiller density was significantly lower in *H. comata* than in *N. viridula* throughout the study (Fig. 2 and Table 2).

Because of its larger basal area, *N. viridula* always had a greater number of potential tiller recruits ($B_{1,2}$, $VT_{1,2}$) per genet than *H. comata*. Average basal area of *N. viridula* genets (206.6 ± 9.8 cm^2) was 3.6× larger than that of *H. comata* genets (56.7 ± 4.2 cm^2; analysis of variance [ANOVA], $F_{1, 237} = 213.29$, $P < 0.0001$). *Nassella viridula* maintained more of its supply of potential tiller recruits than *H. comata* as large juvenile tillers (Fig. 5). In general, primary tillers originated in 2009, secondary tillers originated in 2010, and tertiary tillers originated in 2011. Correspondingly, buds can be aged according to the tiller generation that produced them. Both species maintained supplies of potential tiller recruits throughout the year in the form of mixed-aged bud and juvenile tiller banks originating from multiple annual bud cohorts.

Total tiller density was significantly higher for *H. comata* than for *N. viridula* throughout the study (Fig. 2 and Table 2). *Hesperostipa comata* recruited a large tiller generation ($2VT_{3,4}$) in 2010, when annual precipitation was high (Fig. 3A). Although rainfall was plentiful in 2011, less rain fell in the spring months in 2011 than in 2010, which corresponds to the low recruitment of its next tiller generation ($3VT_{3,4}$). Annual tiller recruitment was more consistent for *N. viridula* than for *H. comata*. Secondary and tertiary tiller generations of *N. viridula* reached a similar density by the fall after their initial recruitment period in 2010 and 2011, respectively (Fig. 2B). Not all primary tillers senesced by the end of 2011 (Fig. 3). Therefore, aboveground tiller longevity may occasionally be >22 mo. Before most secondary tillers transitioned from the small to the large size class in June, low numbers of large secondary tillers existed (Appendix S1) These large secondary tillers could be attached to older primary tillers and may have transitioned from the small to the large size class in the previous spring. As a result, a few 2-yr-old large primary tillers could be maintaining 1-yr-old large secondary tillers in spring 2011.

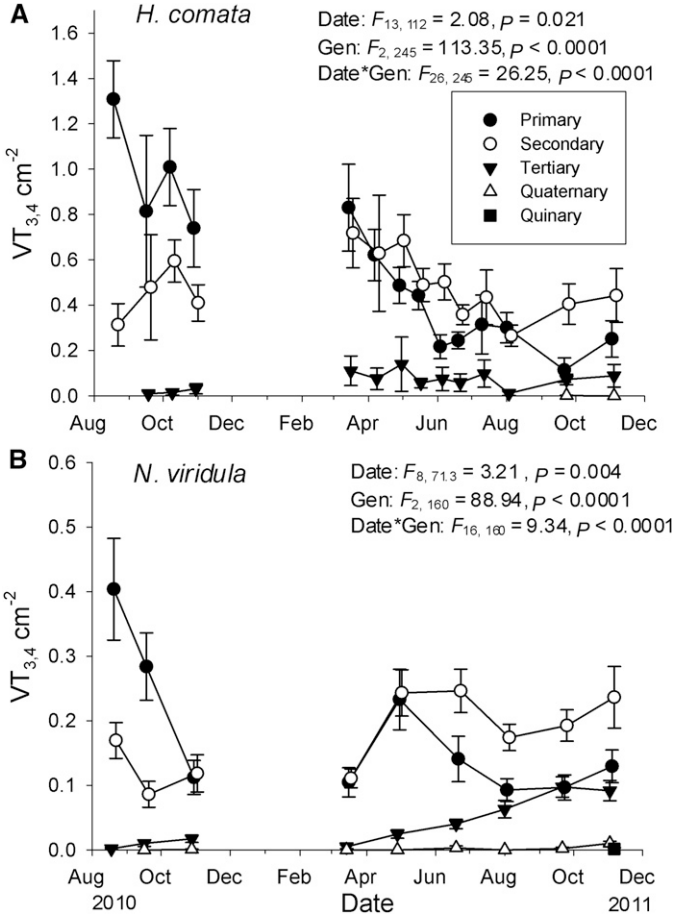

FIGURE 3 Vegetative-tiller ($VT_{3,4}$) density (tillers per genet basal area) according to tiller generation of (A) *Hesperostipa comata* and (B) *Nassella viridula*. Note that the *y*-axis differs between species. Error bars are ±SE.

DISCUSSION

Life-history pattern—*Hesperostipa comata* and *N. viridula* had similar vegetative life cycles. Both species are characterized by genets that live for many years, each composed of multiple overlapping generations of tillers. In both species, the life cycle of a tiller, beginning with a small bud and ending with a flowering or senesced tiller, typically spanned 3 yr (Fig. 6). Recruitment of a new cohort of juvenile tillers began in July and continued into late fall. Juvenile tillers were primarily recruited from buds borne on the tiller generation that had just transitioned the majority of its tillers from the small to the large size class. Beginning in March, overwintered juvenile tillers transitioned into small vegetative tillers as part of a new generation of vegetative tillers. This transition continued until the onset of winter. The majority of a generation's vegetative tillers spent 1 yr in the small (VT_3) size class. In June, these small vegetative tillers (VT_3) transitioned into large vegetative tillers (VT_4). One year later, ~10% of these tillers flowered. Those that did not flower either senesced or lived for another year (Fig. 6, cohort A, arrow extending into growing season 5). In summary, vegetative tillers of both *H. comata* and *N. viridula* can live ≥26 mo because an average, small vegetative tiller will be recruited from a large juvenile tiller in March, transition to a large vegetative tiller 16 mo later in July, and flower or senesce the following spring (Fig. 6, cohort A).

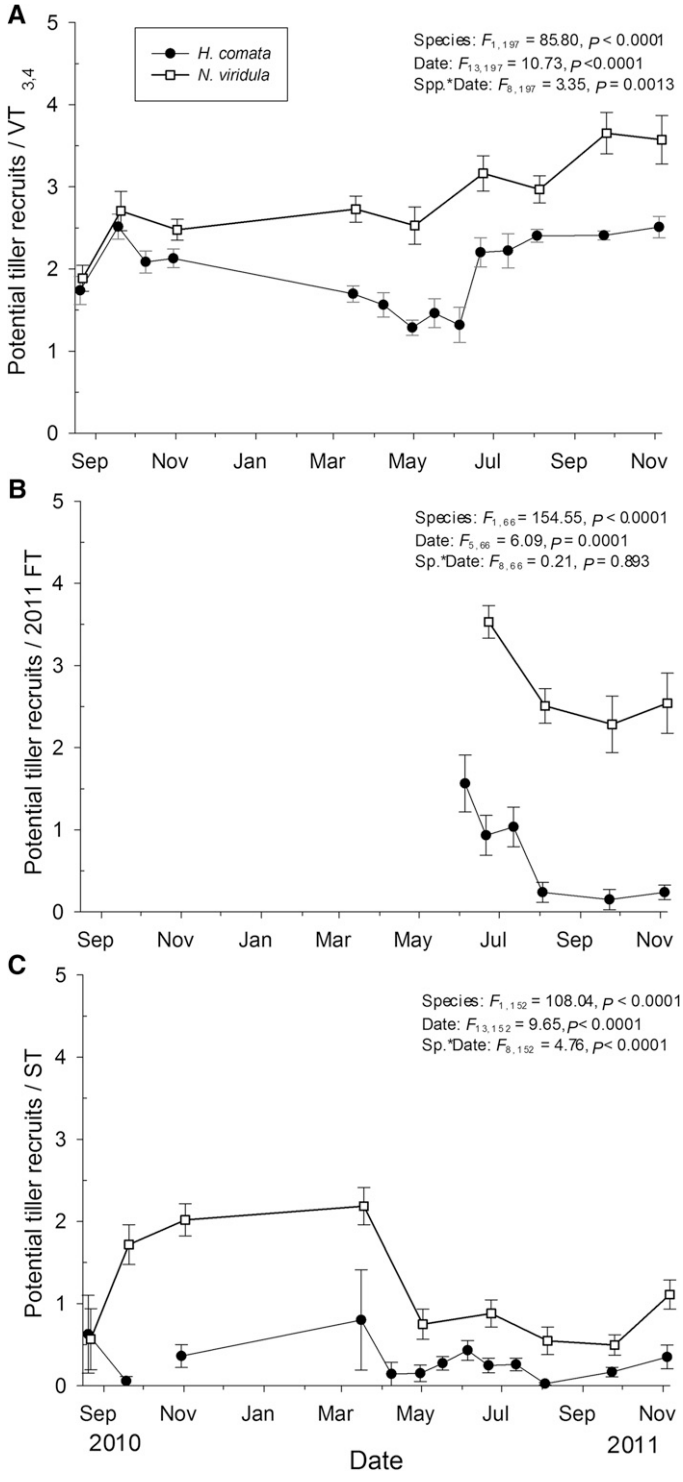

FIGURE 4 Production of potential tiller recruits on (A) vegetative ($VT_{3,4}$), (B) flowering (FT), and (C) senesced tillers (ST) of *Hesperostipa comata* and *Nassella viridula*.

In comparison with the genet bud-bank and tiller characteristics of another C_3 caespitose grass *D. oligosanthes* in tallgrass prairie (Ott and Hartnett, 2012a), *H. comata* and *N. viridula* maintained a similar aboveground cool-season growth phenology, often overwintering in the tiller stage as well as the bud stage. The supply of

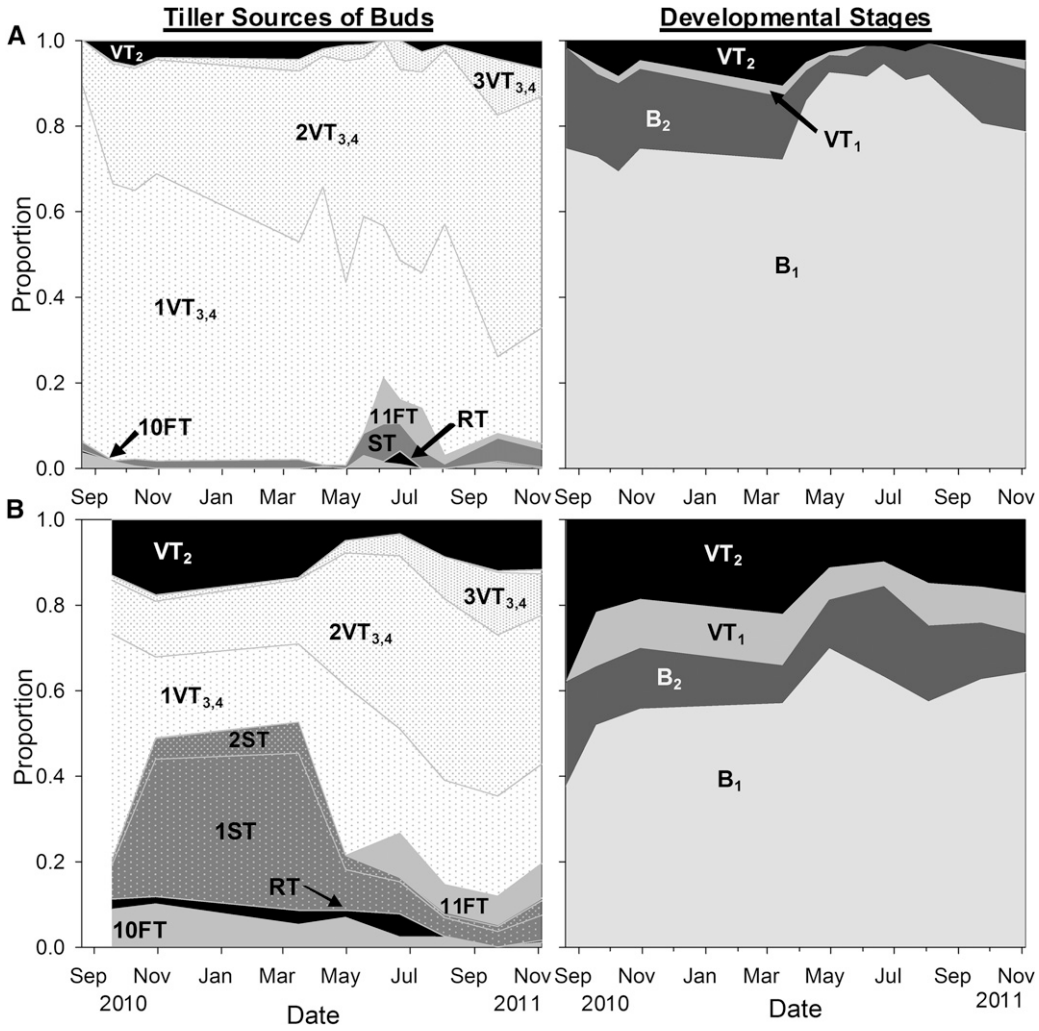

FIGURE 5 Proportions of entire genet's number of potential tiller recruits by tiller source and developmental stage in (A) *Hesperostipa comata* and (B) *Nassella viridula*. Potential tiller recruits could be supported by multiple generations of vegetative (VT_{2-4}), flowering (10FT = 2010 flowering, 11FT = 2011 flowering), senesced (ST), and residual (RT) tillers. Generations are denoted by prefacing numerals. Note that the small, solid white area between $3VT_{3,4}$ and VT_2 represents the portion of potential tiller recruits from $4VT_{3,4}$ and $5VT_{3,4}$; and the small, solid gray area above 2ST in fall 2011 *N. viridula* represents 3ST and 5ST. Bud developmental stages could be classified as small (B_1) or large (B_2), and juvenile tillers could be classified as small (VT_1) or large (VT_2).

potential tiller recruits of *H. comata* and *N. viridula* comprised multiple annual generations, whereas *D. oligosanthes* maintained only a single generation. As a result, buds of *H. comata* and *N. viridula* lived for ≥2 yr, whereas buds of *D. oligosanthes* lived for ~1 yr. However, all three of these caespitose species maintained a supply of potential tiller recruits in multiple stages of development throughout the annual cycle. Multiple developmental stages also occur in the rhizomatous C_3 perennial grass *Pascopyrum smithii* (Ott and Hartnett, 2015). Thus, this continual maintenance of a supply of potential tiller recruits may be a common characteristic of C_3 grasses, regardless of plant grazing history or growth form, and may be an important trait allowing them to respond flexibly to changes in resources or disturbance, or to shifts in seasonal changes in temperature and moisture (Ott, 2014). *Hesperostipa comata* and *N. viridula* usually produced two or three buds per tiller. This bud production per tiller was similar to the lower range of bud production of other dominant and subdominant C_3 caespitose grasses (Mueller and Richards, 1986; Dalgleish et al., 2008; Pelaez et al., 2009; Ott and Hartnett, 2012a) and was very similar to the dominant C_3 caespitose *N. tenuis* in Argentina (Busso et al., 1993).

***Contribution of tiller types to the bud bank*—**Genet growth (tiller population growth) in *H. comata* and *N. viridula* was not meristem limited (as defined in Dalgleish and Hartnett, 2006), in that a large supply of belowground buds for new tiller recruitment was consistently present. Different tiller types contributed unequally to the genet's supply of potential tiller recruits primarily because tiller population size and bud longevity differed among tiller types rather than bud production per tiller. Bud longevity was closely correlated with the aboveground longevity of its parent tiller for most tiller types, although *N. viridula* did maintain buds and juvenile tillers for up to 6 mo after tillers had flowered or senesced. Live vegetative tillers were the main source of potential tiller recruits. However, the pool of potential tiller recruits from these live tillers was undergoing dynamic changes. Potential tiller recruits were lost from the oldest tiller generation as it declined but were added from the youngest tiller generation as its population and tiller size increased, creating a constant refreshing of the pool of potential tiller recruits. Flowering tillers made no significant contribution to new tiller recruitment. Bud production occurs before vegetative tillers transition to flowering tillers (Langer, 1972; Ott and Hartnett, 2011). Buds may be less likely to grow out, once a tiller has transitioned from vegetative to flowering, because of the high allocation of resources to flowering-culm development. However, buds on flowering tillers of the C_4 rhizomatous grass *A. gerardii* were more likely to grow out than buds on vegetative tillers (Ott and Hartnett, 2011).

***Vegetative reproduction characteristics and coexistence*—**Occupancy of different niches, differential demographic responses to disturbance, and fluctuations in resource availability are a few of the mechanisms that enable species coexistence. Niches are often determined by resource utilization/preferences of a species such as microtopography, nutrients, soils, or light conditions. Coexistence of species may also be facilitated by differences in their phenology and life history. In clonal grasses, genet survival and fitness are strongly determined by vegetative reproductive characteristics. In

TABLE 2. Statistical effect of species and sample date on bud and tiller production in the C_3 caespitose grasses *Hesperostipa comata* and *Nassella viridula* from a two-way ANOVA (abbreviations are defined in Table 1). Significant results (at $\alpha = 0.05$) are in bold.

Response variable	Species	Date	Species*date
Buds ($B_{1,2}$) per basal area	**$F_{1, 196} = 18.29$ $P < 0.0001$**	**$F_{13, 196} = 2.06$ $P = 0.018$**	**$F_{8, 196} = 2.18$ $P = 0.031$**
Juvenile tillers ($VT_{1,2}$) per basal area	**$F_{1, 196} = 83.9$ $P < 0.0001$**	**$F_{13, 196} = 4.71$ $P < 0.0001$**	$F_{8, 196} = 1.41$ $P = 0.19$
Vegetative tillers ($VT_{3,4}$) per basal area	**$F_{1, 196} = 52.0$ $P < 0.0001$**	**$F_{13, 196} = 2.86$ $P = 0.0008$**	**$F_{8, 196} = 2.67$ $P = 0.008$**
Senesced tillers (ST) per basal area	$F_{1, 188} = 1.97$ $P = 0.16$	**$F_{13, 188} = 3.04$ $P = 0.0004$**	**$F_{7, 188} = 5.15$ $P < 0.0001$**
Total tillers ($VT_{3,4}$, FT, ST, RT) per basal area	**$F_{1, 196} = 68.0$ $P < 0.0001$**	$F_{13, 196} = 1.15$ $P = 0.32$	**$F_{8, 196} = 3.16$ $P = 0.002$**

general, *H. comata* and *N. viridula* had very similar vegetative reproductive characteristics. Therefore, other factors likely promote their coexistence.

Several subtle vegetative-reproductive and bud-bank differences may enable small *N. viridula* genets to have greater survival than *H. comata*, especially following localized disturbance, drought, or

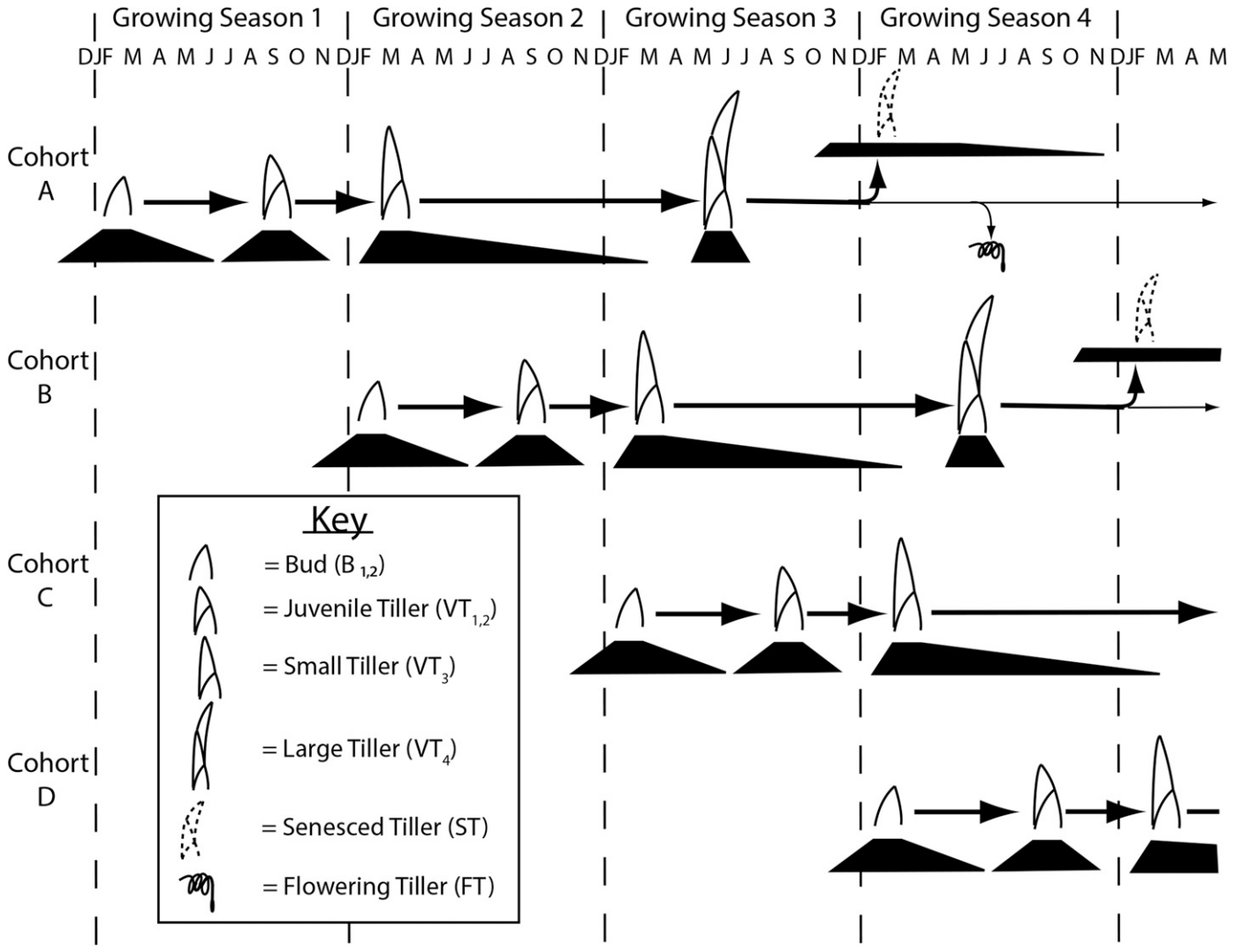

FIGURE 6 Generalized diagram of the life cycles of a tiller for the C_3 caespitose grasses *Hesperostipa comata* and *Nassella viridula*. Months are indicated by the first letter below the growing season. The stage symbol was placed below the month wherein ~50% of the cohort had transitioned to that stage. The black trapezoids beneath each symbol indicate the span of time when bud natality or the specific transition leading to that stage began and ended. Although the majority of tillers follow the general pattern of Cohort A when ungrazed and unburned, there are exceptions in which tillers transitioned to stages at alternative times. The initial bud was assumed to have been borne on a juvenile tiller or a small tiller (VT_3) of the previous cohort (e.g., cohort B's bud was produced by cohort A's small tiller).

herbivory, and support recovery of *N. viridula* from natural fragmentation as genets age. *Nassella viridula* had higher bud production per tiller, higher juvenile tiller density, and greater longevity of buds on senesced and flowering tillers than *H. comata*. The consistent annual tiller recruitment of *N. viridula* would also favor greater genet survival than *H. comata*, especially under heavily fluctuating or poor environmental conditions. The larger basal areas of *N. viridula* genets may be a consequence of their higher survival rate due to these bud and tiller characteristics. By contrast, total tiller density (tillers per unit area) was significantly and consistently higher for *H. comata* genets than for *N. viridula*, which indicates that it may be more efficient in the local monopolization of resources than *N. viridula*.

Nassella viridula genets are likely older than *H. comata* genets. Hollow crown development, a common indicator of an older genet that lowers genet tiller density (Gatsuk et al., 1980) and, hence, its ability to consolidate resources, was often observed in *N. viridula*. Once genets are established with large basal areas, they maintain large numbers of potential tiller recruits per genet, which assist in their continued survival.

***Polycyclic tillers*—**Polycyclic tillers, tillers that live for >1 yr, could enhance the population stability and persistence of perennial grasses. Because multiple annual generations of tillers are alive during the same growing season, genet growth should be buffered against interannual variability in growing conditions, given that years of low tiller recruitment would be ameliorated by years of high tiller recruitment. However, populations of polycyclic species would not retain any buffering capacity in successive years of unfavorable growing conditions characterized by low tiller recruitment. Polycyclic tillers, similar to higher-order bud production, enable caespitose grasses to achieve a dense belowground bud bank (Ott and Hartnett, 2012b). Polycyclic tillers also create mixed-age bud banks composed of multiple annual bud cohorts, each formed under different conditions, which could uniquely influence a bud cohort's survival and/or degree of dormancy. Diversifying the buds in the bud bank and having younger bud cohorts already produced as older bud cohorts die could also buffer tiller population dynamics. This mixed-age bud bank differs from those produced by perennial grasses with annual tillers (e.g., *A. gerardii*; Ott and Hartnett, 2012a), whose older buds no longer have a live parent tiller from which dormancy cues or provisions for bud maintenance may be received. Lag effects, which can affect the long-term stability and persistence of perennial grass populations, can be introduced through polycyclic tillers and long-lived buds. Impacts of precipitation or resource inputs on the current year's bud formation or tiller recruitment might not be evident until 1 or 2 yr later, when new tillers recruited from the buds or that tiller cohort become adult tillers (Fig. 6). Tiller polycyclicity could explain why demographic responses of *H. comata* lagged climate by ≥1 yr (Dalgleish et al., 2011). Although shoot cyclicity has recently been included as a trait in a clonal-growth database and is documented in both European and North American grasses (White, 1977; Jónsdóttir, 1991; Zhang and Romo, 1995; Janisova, 2006; Klimesova and Klimes, 2008), more research is needed to understand how this trait contributes to clonal growth and affects the population dynamics of grasses.

CONCLUSION

Although the size of a genet's bud bank may remain relatively stable, the bud bank is dynamic, undergoing continuous inputs and outputs closely tied with the tiller dynamics of the genet. These two co-occurring C_3 caespitose grasses had generally similar bud and tiller dynamics but differed in their bud production, bud longevity, and tiller-recruitment consistency, which can affect genet survival. Knowledge of the vegetative life-history and bud-bank dynamics of dominant C_3 grasses in the northern Great Plains will help us understand differences of co-occurring species and predict their responses to changing drivers such as fire, grazing, and climatic variability.

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