Table S1. Air temperatures and precipitation near the commercial breeding site on a sandy soil. SD = standard deviation. Average daily temperatures were collected from weather station Woensdrecht (Royal Netherlands Meteorological Institute, 51°27'N, 4°20'E) and precipitation data were collected from weather station Steenbergen (Royal Netherlands Meteorological Institute, 52°32'N, 3°53'E). These stations were located 10.2 and 4.6 km from the site, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Period | temperature (°C) | | precipitation (mm) | |
| mean | SD | sum | SD |
| Mar.–Aug. 2012 | 13.5 | 5.0 | 568 | 7.3 |
| Mar.– Aug. 2013 | 12.4 | 6.7 | 295 | 4.0 |
| Mar.– Aug. 1999–2019 | 13.8 | 5.3 | 424 | 5.1 |

Table S2. Pot experiment. Shoot tissue N concentration and dry matter yield means and standard errors of the mean (SEM) of diploid and tetraploid populations within N fertilisation levels. *P*-values are given for the effect of populations.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | tissue N concentration (g g DM-1) | | | dry matter yield (g m-2) | | |
| population ploidy | N fertilisation  level (g m-2) | mean | SEM | *p*-value | mean | SEM | *p*-value |
| diploid | 0 | 26.5 | 2.25 | 0.647 | 94 | 10.9 | 0.700 |
| 6 | 38.5 | 3.24 | 0.295 | 146 | 10.1 | 0.151 |
| 12 | 47.5 | 2.00 | 0.408 | 166 | 14.2 | 0.516 |
| tetraploid | 0 | 24.1 | 1.69 | 0.063 | 93 | 8.7 | 0.942 |
| 6 | 34.9 | 2.55 | 0.529 | 151 | 9.2 | 0.673 |
| 12 | 48.1 | 1.96 | 0.005 | 174 | 9.4 | 0.265 |

Table S3. Field experiment. Mean shoot tissue water soluble carbohydrate (WSC) and acid detergent fibre (ADF) concentrations, and percentage of *in vitro* digestible organic matter of the control, 2Nlow and 4Nlow populations at all N fertilisation levels, at the first harvest of 2019. SEM = standard error of the mean.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| parameter | control | 2Nlow | 4Nlow | *p*-value population effect | SEM |
| WSC, g kg DM-1 | 146 | 155 | 150 | 0.322 | 5.0 |
| ADF, g kg DM-1 | 255 | 255 | 250 | 0.195 | 2.7 |
| IVDOM, % | 81.5 | 81.3 | 82.0 | 0.125 | 0.17 |

Table S4. Field experiment. Mean shoot tissue water soluble carbohydrate (WSC) and acid detergent fibre (ADF) concentrations, and percentage of *in vitro* digestible organic matter of the control, 2Nlow, 2Nhigh, 4Nlow and 4Nhigh populations at a 25 g m-2 year-1 N fertilisation level, at the first harvest of 2019. SEM = standard error of the mean.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| parameter | control | 2Nlow | 4Nlow | 2Nhigh | 4Nhigh | *p*-value population effect | SEM |
| WSC, g kg DM-1 | 105 | 119 | 124 | 126 | 126 | 0.244 | 3.3 |
| ADF, g kg DM-1 | 274 | 271 | 266 | 259 | 255 | 0.084 | 2.4 |
| IVDOM, % | 81.4 | 82.2 | 82.5 | 82.7 | 83.3 | 0.249 | 0.25 |

Figure S1. Field experiment. Mean tissue N concentration per population at ten consecutive harvests. Concentrations with an unequal superscript differed significantly (p < 0.05). Mean tissue N concentrations per population and N fertilisation level are presented in Figure 2.

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Figure S2. Field experiment. Mean tissue N concentration per population at ten consecutive harvests. Concentrations with an unequal superscript differed significantly (p < 0.05). Mean tissue N concentrations per population and N fertilisation level are presented in Figure 2.

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Table S5. Field experiment. N use efficiency (NUE) and N uptake efficiency (NUptE) of ryegrass populations at ten consecutive harvests.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| year | cut | population | NUE (g DM g N-1) | NUptE (g N g N-1) |
| 2017 | 1 | control | 21.8 | 0.69 |
|  |  | 2Nlow | 22.4 | 0.86 |
|  |  | 4Nlow | 21.5 | 0.88 |
|  |  | *p*-value | 0.605 | 0.076 |
|  | 2 | control | 13.5b | 0.69 |
|  |  | 2Nlow | 17.3a | 0.64 |
|  |  | 4Nlow | 18.1a | 0.89 |
|  |  | *p*-value | 0.031 | 0.065 |
|  | 3 | control | 14.4 | 0.74a |
|  |  | 2Nlow | -2.1 | 0.43b |
|  |  | 4Nlow | 22.2 | 0.29b |
|  |  | *p*-value | 0.105 | <0.001 |
|  | 4 | control | 25.3 | 1.29 |
|  |  | 2Nlow | 28.8 | 0.98 |
|  |  | 4Nlow | 27.7 | 0.89 |
|  |  | *p*-value | 0.715 | 0.067 |
| 2018 | 1 | control | 30.5 | 0.58a |
|  |  | 2Nlow | 31.0 | 0.64b |
|  |  | 4Nlow | 31.9 | 0.48a |
|  |  | *p*-value | 0.453 | 0.003 |
|  | 2 | control | 11.1 | 0.34a |
|  |  | 2Nlow | 29.1 | 0.32a |
|  |  | 4Nlow | 104.4 | 0.06b |
|  |  | *p*-value | 0.265 | 0.001 |
|  | 3 | control | 39.1 | -1.08 |
|  |  | 2Nlow | 34.7 | -1.48 |
|  |  | 4Nlow | 32.6 | -1.54 |
|  |  | *p*-value | 0.505 | 0.085 |
|  | 4 | control | 25.9 | -0.28 |
|  |  | 2Nlow | 20.3 | 0.06 |
|  |  | 4Nlow | 12.0 | -0.12 |
|  |  | *p*-value | 0.896 | 0.217 |
| 2019 | 1 | control | 27.0 | 0.68a |
|  |  | 2Nlow | 27.1 | 0.51b |
|  |  | 4Nlow | 26.4 | 0.50b |
|  |  | *p*-value | 0.965 | 0.001 |

ab values with an unequal superscript differed significantly (*p* < 0.05)

Table S6. Field experiment. Mean shoot tissue N uptake per population and N fertilisation level. Data of 2017 and 2018 are annual figures, and data of 2019 are of the first cut. SE = standard error.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| year | N fertilisation, g m-2 | population | shoot tissue N uptake, g m-2 | |
|  |  |  | mean | SE |
| 2017 | 0 | control | 25.2 | 1.1 |
|  |  | 2Nlow | 26.6 | 2.0 |
|  |  | 4Nlow | 24.7 | 1.9 |
|  | 12.5 | control | 35.5 | 1.1 |
|  |  | 2Nlow | 36.3 | 1.4 |
|  |  | 4Nlow | 34.7 | 1.7 |
|  | 25 | control | 43.9 | 1.4 |
|  |  | 2Nlow | 44.2 | 0.2 |
|  |  | 2Nhigh | 42.3 | 1.2 |
|  |  | 4Nlow | 43.1 | 0.9 |
|  |  | 4Nhigh | 42.5 | 2.1 |
| 2018 | 0 | control | 25.4 | 0.7 |
|  |  | 2Nlow | 26.7 | 1.0 |
|  |  | 4Nlow | 27.9 | 0.5 |
|  | 12.5 | control | 25.8 | 0.6 |
|  |  | 2Nlow | 26.7 | 0.9 |
|  |  | 4Nlow | 25.2 | 0.6 |
|  | 25 | control | 28.5 | 0.8 |
|  |  | 2Nhigh | 27.5 | 0.6 |
|  |  | 2Nlow | 29.7 | 1.8 |
|  |  | 4Nhigh | 29.0 | 0.3 |
|  |  | 4Nlow | 27.7 | 0.2 |
| 2019 | 0 | control | 5.9 | 0.3 |
|  |  | 2Nlow | 6.3 | 0.4 |
|  |  | 4Nlow | 5.8 | 0.3 |
|  | 5 | control | 9.0 | 0.4 |
|  |  | 2Nlow | 8.9 | 0.5 |
|  |  | 4Nlow | 8.0 | 0.2 |
|  | 10 | control | 13.4 | 0.2 |
|  |  | 2Nlow | 11.4 | 1.1 |
|  |  | 2Nhigh | 10.5 | 0.7 |
|  |  | 4Nlow | 11.5 | 0.8 |
|  |  | 4Nhigh | 10.2 | 0.9 |

Table S7. Field experiment. Visually estimated soil coverage by perennial ryegrass as a percentage of the total soil surface, per population and N fertilisation level. SEM = standard error of mean.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| year | 2018 | | | 0 | 2019 | | |
| N fertilisation,  g m-2 year-1 | 0 | 12.5 | 25 |  | 0 | 12.5 | 25 |
| population |  |  |  |  |  |  |  |
| control | 85.0 | 89.9 | 90.9 |  | 80.8 | 86.6 | 91.3 |
| 2Nlow | 86.9 | 90.2 | 89.9 |  | 80.9 | 87.3 | 90.6 |
| 4Nlow | 84.3 | 90.4 | 89.9 |  | 77.5 | 87.3 | 90.1 |
| 2Nhigh |  |  | 90.9 |  |  |  | 90.9 |
| 4Nhigh |  |  | 89.4 |  |  |  | 91.1 |
| *p*-value | 0.609 | 0.974 | 0.889 |  | 0.051 | 0.667 | 0.873 |
| SEM | 1.12 | 0.78 | 0.53 |  | 0.68 | 0.38 | 0.34 |